JOURNAL OF DAIRY SCIENCE

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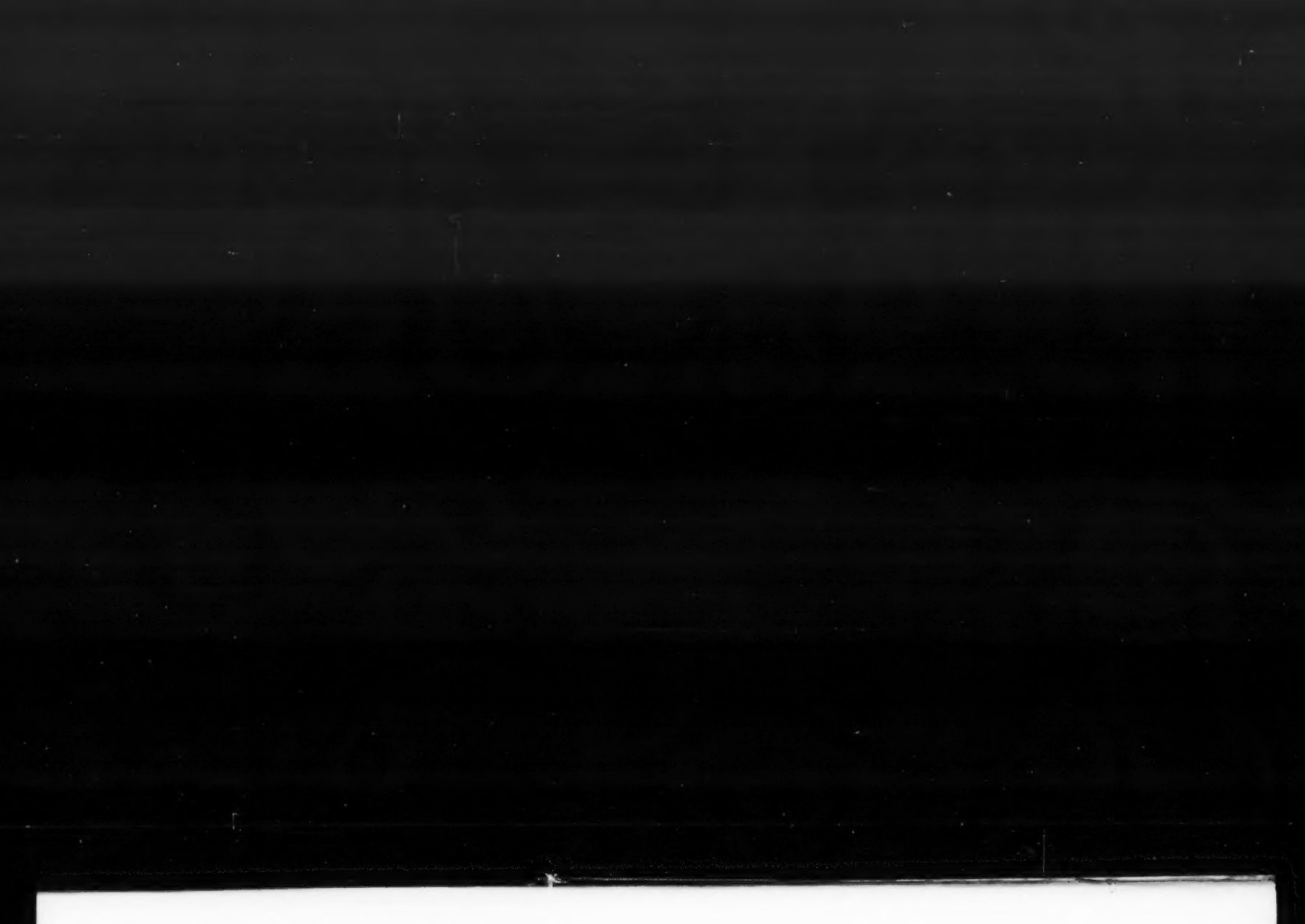

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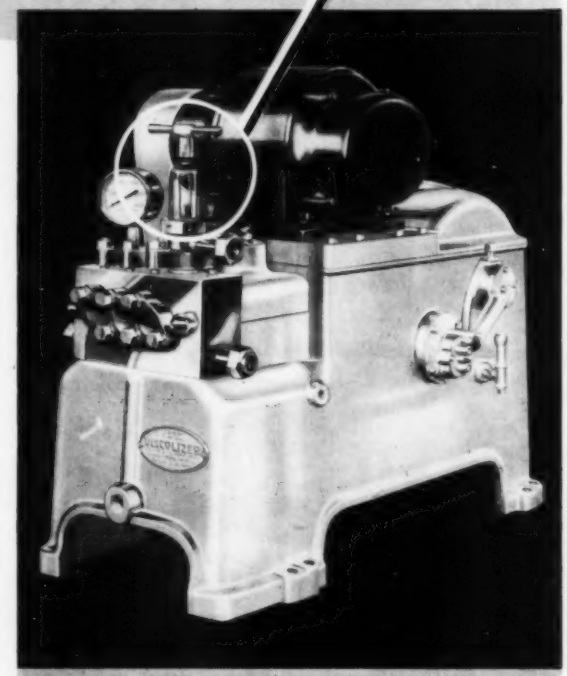

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JOURNAL OF DAIRY SCIENCE

VOLUME XXIII APRIL, 1940 NUMBER 4

ACID VALUES AND ACID RATIOS AS RELATED TO THE KEEPING QUALITY OF SALTED BUTTER[1]

H. A. BENDIXEN

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Since acidity affects the activity of micro-organisms and enzymes as well as the speed of purely chemical reactions, it would seem that the amount of acid present or developing in butter when held for one week at 21° C. might give an indication of the keeping quality of the butter. It was realized that the influences of acidity may exert themselves in different directions, but in order to study the realtionships which might most commonly exist between acidity and the keeping quality of salted butter, especially sweet cream butter, when held for one month at 0–5° C., a number of samples of commercial butter obtained from various Washington creameries were examined for the acid values of both the butter and the butterfat and for the ratio of butterfat acidity to butter acidity. The latter will be referred to as the acid ratio.

REVIEW OF LITERATURE

That various undesirable bacteria are retarded in their growth by the presence of considerable amounts of acid in butter has been shown by many investigations. Thus, Collins and Hammer (5) when growing lipolytic bacteria on Nile-blue sulphate agar of pH values of 5.3, 6.7, and 7.8 noted that with many of the organisms the most alkaline reaction seemed to favor the hydrolysis of simple triglycerides and natural fats. Grimes (13) concluded that the acidity of ripened butter inhibited the growth of proteolytic bacteria. Sadler and Vollum (26) showed that much more deterioration occurred in butter made from overneutralized cream than in butter from cream of 0.25 per cent acidity or over when inoculated with the bacteria obtained from deteriorated butter. Guthrie, Scheib, and Stark (15) found no significant numbers of proteolytic and lipolytic bacteria in sour cream butter. On the other hand yeast and mold growth is known to be favored by acidity.

Most enzymes seem to be less active in the presence of acid. Thus, Davies

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(6) states that the acidity of ripened cream prevents lipase action even in raw cream. Dorner and Widmer (7) found that the enzyme lipase was inhibited by an acid reaction and according to Babcock, Russell, and Vivian (2) galactase also is retarded by acidity. Davies (6) and Rogers (23) reported the enzyme galactase to be most active in the pH range of 6.4–7.2 at temperatures of 37–42° C. The most active proteolytic enzymes were found by Spitzer, Parfitt, and Epple (28) to be produced by *Bacillus ichthyosmius* and *Achromobacter putrefaciens* and these enzymes seemed to be most active at a pH of 7.0 and greatly reduced in activity at pH 4.0 and 3.0. According to Davies (6) catalase is also slightly inhibited by acid conditions while peroxidase is active over a wide range of pH. However, he states that the inactivation of catalase by heat seems to be retarded by acidity and Zilva (29) found this to be true also in the case of peroxidase. Guthrie, Scheib, and Stark (15) concluded that the action of all enzymes was inhibited by a pH of 4.4–4.64 in unsalted butter.

In the light of the above-mentioned investigations, it remains very doubtful, however, whether the normally low acidity of sweet cream butter exerts any significant retarding effect on the action of undesirable bacteria or enzymes. On the other hand, an acidity which is comparatively high for sweet cream butter may be a general indication of high bacterial contamination.

Rogers and Gray (24) decided that the deleterious effect of lactic acid in producing fishy butter was not due to any organism, enzyme or other substance which would be destroyed by pasteurization at 77–82° C. by the flash method or at 70° C. for 10 minutes, but that acidity itself was responsible. Dyer (8) showed that the development of undesirable flavors in butter held in cold storage at – 17.8° C. was due to the oxidation of non-fatty substances in the butter and that the extent of this chemical change was directly proportional to the quantity of acid present in the cream from which the butter was made. Greenbank and Holm (12) concluded that increases in the acidity of fat increase its susceptibility to oxidation. That the production of trimethylamine, the cause of fishy flavors in butter, is accelerated by the presence of acid was shown by Sommer and Smit (27).

According to Loftus-Hills, Scharp, and Bellair (18) high acidity and a low pH correlated rather closely with low keeping quality in sweet cream butter. Similar results were obtained by Arup and Gilmour (1), Gilmour (10), and Gilmour and Arup (11). The latter investigators concluded that a consideration of pH values was more valuable in selecting butter for cold storage than a knowledge of the acid values, and that butter with a pH of over 6.7 kept better than butter of a lower pH.

Bouska (4) stated that butter tends to be low in keeping quality at a pH of less than 6.0, keeps better at a pH of 6.0–6.8, develops surface flavors readily at pH 7.0, and tallowy flavors at a higher pH.

The results of Patrick, Leighton, and Bisbee (21), Patrick, Leighton,

and Heileman (22), Rogers, Thompson, and Keithley (25), Mortensen (19), and Grimes (13) (14) all indicated that the butter of lowest acidity, whether made from raw or pasteurized cream, had the best keeping quality at various storage temperatures up to 10° C. Larson, Lund, and Miller (17) noticed that the percentage of acid found in the fat increases with the age and rancidity of the butter. Frielinghaus (9) examined many samples of butter for their acid ratio which is the ratio of the acid value of the butterfat to the acid value of the butter. He found that the acid ratio of butter made from cream inoculated with yeasts gradually increased during storage of the butter for 20 days at 4° C. At the same time the flavor became increasingly stronger. He obtained similar results when inoculating the cream with cladosporium and concluded that in the development of rancid flavors the acid ratio will increase, while in the development of high acid flavors it will decrease. When tallowy flavors developed, both the acid values of the butter and of the butterfat were raised, leaving the acid ratio little changed.

METHODS USED

Acid values were determined on 28 samples of sweet cream butter, 51 samples of neutralized cream butter without butter culture, and 8 samples of neutralized cream butter with butter culture, and also on the butterfat separated from these samples. The determinations were made when the samples were fresh, after a week at 21° C., and after a month at 0–5° C.

Acid values of butter

The butter was prepared for titrating by macerating 70–80 grams in a clean, dry cup by means of a spatula until it appeared glossy and salvy.

A 10-gram sample was weighed into a tared porcelain casserole or Erlenmeyer flask on a torsion butter moisture balance. Then 50 ml. of ethyl alcohol, neutral to phenolphthalein, and a few drops of phenolphthalein were added and the mixture brought to a boil on an electric hot plate. The boiling hot mixture was titrated with N/50 NaOH while being stirred constantly. The number of milliliters of N/50 NaOH used to produce a pink color stable for at least one minute was taken as the acid value. This is essentially the method recommended by Bird and Breazeale (3).

Acid values of butterfat

The butterfat was obtained by filling a 50 ml. centrifuge tube with butter, melting it in a water bath at 40–45° C. and centrifuging the tube of melted butter. A sample of 10 grams of the unfiltered oil was titrated immediately by the procedure described above for butter in order to determine its acid value.

Acid ratios

The acid ratios were determined by dividing the acid values of the butterfat by those of the butter and multiplying by 100.

pH values of butter serum

The pH of the serum was determined at 25° C. by means of a Leeds and Northrup quinhydrone pH indicator (catalog No. 7654), using a rectangular plate gold electrode together with a saturated calomel half-cell and a saturated KCl agar bridge. The butter serum was obtained by centrifuging the butter melted at 40–45° C. and removing the butterfat.

Table 1 gives the average, maximum and minimum acid values converted into percentages of acid, calculated as lactic acid. The maximum acidity

TABLE 1

Maximum, minimum and average acidities found in butter and butterfat and calculated from the acid values as percentages of lactic acid

	Per cent acidity calculated as lactic acid in						H ion concentration (as pH)
	Butter			Butterfat			Serum
	When fresh	After 1 week at 21° C.	After 1 month at 0–5° C.	When fresh	After 1 week at 21° C.	After 1 month at 0–5° C.	When fresh
I. Sweet cream butter (28 samples)							
Maximum	0.170	0.203	0.169	0.115	0.152	0.131	5.8
Maximum*	0.130	0.145	0.131	0.113	0.115	0.112	6.0
Minimum	0.070	0.072	0.079	0.038	0.045	0.051	6.8
Average*	0.095	0.109	0.103	0.067	0.076	0.078	6.42
II. Neutralized cream butter without butter culture (51 samples)							
Maximum	0.178	0.207	0.176	0.129	0.203	0.152	6.0
Minimum	0.072	0.079	0.083	0.038	0.050	0.063	7.6
Average	0.122	0.140	0.132	0.084	0.102	0.099	6.50
III. Neutralized cream butter with butter culture (8 samples)							
Maximum	0.160	0.198	0.167	0.162	0.182	0.157	5.6
Minimum	0.110	0.119	0.144	0.070	0.065	0.098	7.0
Average	0.138	0.158	0.155	0.096	0.116	0.125	6.33

* Considering only the 26 samples with pH values of 6.0 or over.

found in fresh sweet cream butter was 0.130 per cent, and in the butterfat of such butter 0.113 per cent. The maximum acidity found in these samples after storage, either for 1 week at 21° C. or for 1 month at 0–5° C. was 0.145 per cent in the butter and 0.115 per cent in the butterfat. The minimum acidity encountered was 0.070 per cent in this type of butter and 0.038 per cent in the butterfat.

Sweet cream butter had a lower minimum acidity and also a lower average acidity than the neutralized cream butter, even though the maximum pH found for sweet cream butter was 6.8 and for neutralized cream butter 7.6. Such observations harmonize with the results of Hunziker and Cordes (16) who found that sweet cream produced butter of a decidedly

lower pH than the same cream when soured, neutralized and then churned at the same acidity as the sweet cream.

Nissen (20) reported the titratable acidity of butter in an aqueous mixture to range from 0.02 to 0.04 per cent, if made from cream of about 0.15 per cent acidity, and from 0.03 to 0.05 per cent if made from cream testing 0.25 per cent in acidity. The values in an alcoholic mixture are always considerably higher than those in an aqueous mixture, probably because of the release of fatty acids from the fat by the alcohol. The pH at the end point of the butter titration in alcohol as conducted in this study usually was 7.8–7.95 compared with 7.35–7.73 as reported by Nissen in his titrations in water.

Acid values and keeping quality

An examination of the data obtained in this study revealed no close relationship between the individual acid values of the fresh butter, the fresh butterfat, or either of these after storage for 1 month at 0–5° C. or for 1

TABLE 2

The increase in the acid value of butterfat during 1 week at 21° C. as related to the keeping quality of salted butter when held for 1 month at 0–5° C.

Number of samples	Increase in acid value of butterfat during 1 week at 21° C.	Average loss in score during 1 month at 0–5° C.	Average score after 1 month at 0–5° C.
	I. Sweet cream butter		
6	Below–0	0.58	36.42
8	0 –0.45	1.06	36.00
6	0.5–0.95	1.67	35.67
5	1.0–1.45	1.70	35.70
1	1.5–1.95	2.00	35.50
2	2.0–2.45	1.75	34.75
28	Average 0.54	1.29	35.86
	II. Neutralized cream butter without butter culture		
5	Below–0	0.40	35.20
12	0 –0.45	0.33	35.17
11	0.5–0.95	0.32	35.00
9	1.0–1.45	0.89	34.67
8	1.5–1.95	0.89	34.88
4	2.0–2.45	0.25	35.13
2	2.5 and over	1.00	34.50
51	Average 1.01	0.51	34.97
	III. Neutralized cream butter with butter culture		
2	Below–0	0	33.50
1	0 –0.45	0.50	36.00
2	0.5–0.95	0	34.75
1	1.0–1.45	1.00	35.00
1	1.5–1.95	0.50	35.00
0	2.0–2.45		
1	2.5 and over	1.00	33.50
8	Average 1.06	0.38	34.25

week at 21° C. on the one hand and loss of score during storage or the score itself after storage on the other hand. A definite trend towards reduced keeping quality was noticed with high increases in the acid values of the butter, and especially the butterfat after storage for a week at 21° C. Table 2 brings out this relationship for the three types of butter. The trend was most pronounced with sweet cream butter, but perceptible also for neutralized cream butter without butter culture. The number of samples of neutralized cream butter with butter culture is too small to warrant conclusions.

Since the deterioration of the butter during storage, especially in the case of sweet cream butter, very often seemed to be in the nature of fat deterioration as judged from the criticisms of the judges, and since Frielinghaus (9) noted that the development of rancidity is accompanied by a significant increase in the ratio of butterfat acidity to butter acidity, the relationship of this ratio to the keeping quality of the samples was studied.

Table 3 indicates that the average acid ratios for each type of butter,

TABLE 3

The acid ratio of salted butter as related to the loss in flavor score of such butter when held for 1 month at 0–5° C.

Number of samples	The average acid ratio in the butter					
	Loss in score during 1 month at 0–5° C.	When fresh	After 1 week at 21° C.	Increase during week	After 1 month at 0–5° C.	Increase during month
	I. Sweet cream butter					
9	2.0– 2.5	62.4	72.1	9.7	81.0	18.6
12	1.0– 1.5	70.8	67.7	– 3.1	74.9	4.1
6	0 – 0.5	71.8	66.7	– 5.1	74.0	2.2
1	– 1.0– – 0.5*	73.8	63.6	– 10.2	60.6	– 13.2
28	Average 1.29	68.4	68.8	0.4	76.3	7.9
	II. Neutralized cream butter without butter culture					
2	2.0– 2.5	78.9	81.2	2.4	85.3	6.4
13	1.0– 1.5	71.3	76.8	5.5	83.8	11.9
29	0 – 0.5	66.8	69.0	2.2	72.1	3.4
7	– 1.0– – 0.5	64.8	71.0	6.1	74.5	9.9
51	Average 0.51	68.2	71.7	3.5	75.7	7.5
	III. Neutralized cream butter with butter culture					
2	1.0– 1.5	83.7	93.9	10.3	93.6	10.0
4	0 – 0.5	67.3	68.6	1.3	73.9	10.3
2	– 1.0– – 0.5	60.6	55.4	– 5.3	67.3	10.0
8	Average 0.38	69.7	71.6	1.9	80.4	10.7

when fresh, after 1 week at 21° C., and after 1 month at 0–5° C. were generally larger as the losses in flavor score increased. The only exception was in the fresh sweet cream butter where higher average acid ratios were found associated with the best keeping butter. However, after a week and

after a month, this relationship was reversed so that a large increase in the average acid ratio was associated with large score losses while decreases in the average acid ratio were associated with small score losses. For instance, in the samples which lost 2.0 to 2.5 points in flavor score during storage, the butterfat acidity was on the average 62.4 per cent of the butter acidity in the fresh butter, and this percentage increased to 72.1 after 1 week at 21° C. and to 81.0 after 1 month at 0–5° C. In the samples which lost little or even gained in score during storage, the average acid ratio decreased or increased only very slightly during storage. The smaller the loss in score was during storage the greater was the average decrease in acid ratio. Thus the keeping quality of sweet cream butter was increased when the acidity of the fat increased at a slower rate than the acidity of the butter. This relationship was not quite as apparent in the samples of neutralized cream butter but held true for the few samples of neutralized butter made with butter culture.

TABLE 4

The change in the acid ratio of salted butter during storage at 21° C. and 0–5° C. as related to the keeping quality of such butter when held for 1 month at 0–5° C.

Number of samples	Increase in acid ratio during 1 week at 21° C.		Loss in score during 1 month at 0–5° C.	Score after 1 month at 0–5° C.
	Range	Average	Average	Average
	I. Sweet cream butter			
10	− 5.0 and less	−11.80	0.90	36.25
5	− 4.9 to − 0.1	− 2.36	0.90	35.80
4	0 to 4.9	2.10	1.63	35.63
9	5.0 and over	14.54	1.78	35.56
15	−24.2 to − 1.2	− 8.65	0.90	36.10
13	0.2 to 39.1	10.72	1.73	35.58
	II. Neutralized cream butter without butter culture			
16	−39.6 to − 1.7	−10.81	0.59	35.19
35	0.1 to 34.7	10.18	0.47	34.50
	III. Neutralized cream butter with butter culture			
5	− 9.4 to − 1.1	− 6.80	0.20	34.50
3	4.8 to 29.8	16.37	0.67	34.50
	Increase in acid ratio during 1 month at 0–5° C.			
	range	average		
	I. Sweet cream butter			
10	−20.3 to − 0.7	− 8.33	0.95	36.20
16	1.1 to 42.7	19.34	1.56	35.69
	II. Neutralized cream butter without butter culture			
15	−43.8 to − 0.2	− 7.70	0.20	35.27
29	1.2 to 42.6	13.62	0.64	34.91

To show still more distinctly the effect of the change of the acid ratio on the keeping quality of salted butter table 4 is presented. It indicates that when the acid ratio in sweet cream butter decreased during 1 week at 21° C., the average loss in flavor score during storage at 0–5° C. for 1 month was only about half as great as when the ratio increased. Furthermore, the average score itself after storage was distinctly higher when the acid ratio decreased than when it increased. In the case of neutralized cream butter, the average loss in score under these conditions was nearly the same, although the actual score after storage averaged somewhat higher.

In the case of butter made from neutralized cream using butter culture, the average loss in score was smaller but the average final score was the same, when the acid ratio decreased, as compared with an acid ratio increase.

Considering the effect of the change in acid ratio on the keeping quality of individual samples, it was found that of the 9 samples of sweet cream butter which lost 2.0–2.5 points in score during storage 8 showed an increase in the acid ratios during 1 week at 21° C. and of 13 samples which lost 1 point or less in storage 10 samples showed a decrease in the acid ratio during the week at 21° C. Thus a knowledge of the change in the acid ratio seems to offer a valuable aid in foretelling keeping quality, at least for sweet cream butter. Although the acid ratio itself after 1 week at 21° C. is of some value for this purpose, it is not as significant as the change in ratio.

The average acid ratio for each type of butter studied was higher after storage than when fresh and higher after 1 month at 0–5° C. than after 1 week at 21° C. The figures are shown in table 3. Table 4 also brings out the relationship of the change in the acid ratio during a month at 0–5° C. to the keeping quality of the butter. Here an increase in the acid ratio is shown to be associated with a marked decrease in keeping quality of both sweet cream and neutralized cream butter.

A study of individual samples revealed that in 22 of the 26 sweet cream butter samples, 37 of the 44 neutralized samples and in 4 of the 5 cultured samples for which complete data were available, the acid ratio changed in the same direction during the week at 21° C. as during the month at 0–5° C.

DISCUSSION

It seems that no correlations exist between acid values and keeping quality which are definite enough to enable the prediction of keeping quality for every individual sample of butter. At the same time, certain trends were noticed which deserve attention when attempting to predict the keeping quality of salted butter, especially sweet cream butter. Furthermore, since the nature of butter deterioration may vary greatly, and since the accuracy of butter scoring standards is far from what is desirable, such

general trends, as were observed in this study, may have considerable significance.

On the basis of the observations made here on the acid values of butter and butterfat, it seems that the most valuable aid in the prediction of the keeping quality of sweet cream butter is the change, during 1 week at 21° C., in the percentage of the butter acidity which is closely associated with the butterfat. When this acid ratio increased during 1 week at 21° C. the loss in score during storage for 1 month at 0–5° C. was considerably greater, as a general rule, than when the ratio decreased. Acidity develops independently in butter and in butterfat during storage and it is the acidity increase of the fat in relation to the acidity increase of the butter taking place during storage, which appears to be a significant indicator of the keeping quality of butter when stored for 1 month at 0–5° C.

The acid ratio itself after 1 week at 21° C. also averaged higher in the butter of the poorer keeping qualities. Furthermore there was a definite trend toward reduced keeping quality in salted butter with increases in the butter acidity, and especially the butterfat acidity during 1 week at 21° C. However, the acid values themselves, either in the butter or in the butterfat, when fresh and after 1 week at 21° C. apparently are of less significance as an index of keeping quality.

SUMMARY

The acid content, calculated as lactic acid, and as determined in this investigation for 26 samples of sweet cream butter with pH values above 6.0 was 0.07–0.13 per cent when fresh, 0.072–0.145 per cent after 1 week at 21° C., and 0.079–0.131 per cent after 1 month at 0–5° C. It averaged 0.096 per cent when fresh, 0.109 per cent after 1 week at 21° C. and 0.103 per cent after 1 month at 0–5° C. For 51 samples of neutralized cream butter these averages were 0.122, 0.140 and 0.132 per cent, respectively.

High acid values of butter and butterfat before and after storage showed a slight tendency toward reduced keeping quality. High increases in the acid values after storage at 21° C. and 0–5° C. did show a fairly close correlation with reduced keeping quality of sweet cream and neutralized cream butter but especially of sweet cream butter.

An increase in the acid ratio (fat acidity: butter acidity) during 1 week at 21° C. and during 1 month at 0–5° C. seemed to be closely related to poor keeping quality, especially in the case of sweet cream butter. The average acid ratios were higher after 1 month at 0–5° C. than after 1 week at 21° C.

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THE DENSITY AT 140° F. OF THE MATERIALS EXPRESSED AS FAT BY VARIOUS VOLUMETRIC TESTS OF CREAM*

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The density of the material comprising the fat columns in the various volumetric tests has received very little attention. This is undoubtedly due to the fact that the procedures of the tests have usually been adjusted so that the results will agree with the official method. There has been some investigation as to the absolute accuracy of some of the rapid methods of testing dairy products for fat. The Babcock test bottles are calibrated for fat of a definite density at a definite temperature. Several modifications of the Babcock procedure have been proposed. It seems obvious that a modification using alcohol would give a fat column of a density different from that of a test using acid. Babcock (1) in devising his test bottle assumed the specific gravity of the fat to be 0.9000 at 120° F. He did not believe that a variation in temperature of reading from 110 to 150° F. would materially affect the results for milk although he recommended using the higher temperature. The difference for the 40 degree range was given as being less than 0.1 per cent for a 5 per cent milk test. In 1891 Farrington (2) obtained the greatest accuracy compared to gravimetric methods if the fat column in the Babcock milk test was read at 140° F.

The directions for testing cream were likewise lax in spite of the greater differences that were bound to occur. Webster (3) in 1904 reported the density of fat to be 0.9004 at 100° F., but by weighing a definite amount of fat into a test bottle he was able to account for all of it only by reading at 120° F. A little later Hunziker, Spitzer, Mills and Crane (4) insisted that the correct reading temperature was 135° F. for that was the temperature at which the fat had a specific gravity of 0.9000. Then Ross and McInerney (5) recommended a reading temperature between 140° and 150° F., obtaining better checks with ether extraction at 150° F., but for milk they reported that there was no difference by reading at either 100° or 146° F. Bailey (6) disagreed with all previous investigators when he reported that all density measurements at Iowa State College showed the density of butterfat to be about 0.8974 at 120° F. He states that the density varies by 0.00038 per degree F. This gives a density of 0.9000 at about 113° F. Hepburn (7) used a temperature of 125 to 130° F. for his modified Babcock test for butter. Dahlberg (8) found the specific gravity to be 0.8943 at 132° F., however he

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read at a temperature of 132° F. and reported that the Babcock bottle was calibrated for 0.9000 at 135° F. Tracy and Overman (9) recommended reading the test at the now commonly accepted temperature of 135–140° F. Some other investigators gave the following specific gravity values for butterfat: Storch (10) 0.9335 at 15° C. (59° F.), Rahn (11) 0.89857–0.89729 at 50° C. (122° F.), and Koestler (12) 0.9355–0.9448 at 15° C. (59° F.).

The data reported by various investigators on the density of butterfat may be summarized as follows:

Investigator	Density of fat reported	Read Babcock test at
Babcock (1)	0.9000 at 120° F.	110 to 150° F.
Farrington (2)		140° F.
Webster (3)	0.9004 at 100° F.	120° F.
Hunziker and coworkers (4)	0.9000 at 135° F.	135° F.
Ross and McInerney (5)		140 to 150° F.
Bailey (6)	0.8974 at 120° F.	130° F.
	0.9000 at 113° F.	
Hepburn (7)		125 to 130° F.
Dahlberg (8)	0.8943 at 132° F.	132° F.
Tracy and Overman (9)		135 to 140° F.
Storch (10)	0.9335 at 15° C. (59° F.)	
Rahn (11)	0.89857 to 0.89729 at 50° C. (122° F.)	
Koestler (12)	0.9355 to 0.9448 at 15° C. (59° F.)	

Variations in the composition of butterfat due to feed and other factors may partially explain such conflicting results.

EXPERIMENTAL

Cream with a fat content of about 35 per cent obtained from mixed herd milk during the late fall and winter was tested by the Mojonnier method, by the Babcock method and by the following modifications of the Babcock method: the Minnesota 202, the Minnesota Nafis, the N-butyl alcohol, and the amyl alcohol. The Minnesota 202 refers to the original Minnesota Babcock Test reagent and the Minnesota Nafis to the reagent as sold by the Nafis Company. In the N-butyl alcohol test 1 ml. of N-butyl alcohol was used in addition to sulfuric acid to 9 gr. of cream. In the amyl alcohol test 2 ml. of amyl alcohol and about 17 ml. of sulfuric acid was used to 9 gr. of cream to give about the same proportion as in the Gerber test.

Picnometers having a volume of about 1 ml. were calibrated with mercury and used in determining the density of the material read as fat in the various tests. As check samples a small portion of the cream was churned and the resulting butter melted and filtered to obtain practically pure butterfat. The results of these determinations are summarized in tables 1 and 2. A marked variation was found to exist in the density of the fatty materials from the various tests when measured at 140° F. At this temperature the pure

butterfat had an average density of 0.89169; the Babcock fat column, 0.89512; the Minnesota Nafis fat column, 0.89085; and the Minnesota 202 fat column, 0.88398. Calculating the per cent overreading, which theoretically would occur due to the density not being exactly 0.9000 at 140° F., gave an error of 0.932 per cent that would be encountered even if the fat columns were pure fat. Similarly the calculated error due to the specific gravity of the material read as fat at 140° F. amounted to 0.545 per cent for the Babcock, 0.825 per cent for the N-butyl alcohol, 0.966 per cent for the amyl alcohol, 1.027 per cent for the Minnesota Nafis, and 1.812 per cent for the Minnesota 202 test.

TABLE 1

The density at 60° C. (140° F.) of the materials read as fat with the various tests of cream

Determination date	Filtered butterfat	Mojonnier	Babcock	Nafis	202	N-butyl	Amyl
10/7/35		.89410	.89359	.89036	.88406		.89065
10/21/35	.89261	.89252		.89065	.88299	.89056	.89136
	.89400	.89410	.89644	.89185	.88456	.89288	.89232
1/18/36	.89199	.89341	.89403	.88994	.88433	.89368	.89029
	.88998	.89322	.89643	.89148	.88400	.89344	.89241
Mean	.89169	.89347	.89512	.89085	.88398	.89264	.89139

Bailey (6) reported that the density of butterfat varied by 0.00038 per degree Fahrenheit. Using this value it was calculated that the fatty materials studied would have an approximate density of 0.9000 at 118° F. for pure butterfat, at 127° F. for the Babcock test fat column, 116° F. for the Minnesota Nafis test, 72° F. for the Minnesota 202 test, 117° F. for the amyl alcohol test and 121° F. for the N-butyl alcohol test. The effect of impurities in the fat columns of the various tests would probably change the above calculated values.

The higher density of the Babcock test fat columns over pure butterfat was probably due to the inclusion of water, while in the case of the 202 test

TABLE 2

The calculated error due to reading the various fat columns at 140° F., and the calculated temperature at which the fat columns would have a density of 0.9000

Testing method	Error at 140° F.	Temperature at which* density would be approx. 0.9000
	per cent	*° F.*
Pure butterfat	+0.932	118
Babcock	+0.545	127
Nafis	+1.027	116
202	+1.812	72
N-butyl	+0.825	121
Amyl	+0.966	117

* Assuming the density of fat varies by 0.00038 per degree F. (6).

the lower density was probably due to alcohol. The presence of these impurities in the fat column introduces a fundamental error.

The results here reported indicate that interesting results may be obtained by conducting further work to determine the proper temperature at which to read the various tests.

SUMMARY

1. The density of the materials read as fat with the various volumetric tests was less than 0.9000 at 140° F.

2. The error in the volumetric tests due to variation in the density at 140° F. of the materials read as fat was the least with the Babcock test (about 0.55 per cent) followed in order by the N-butyl alcohol, the amyl alcohol, the Minnesota Nafis and the Minnesota 202 test (over 1.8 per cent).

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REDUCTION OF CURD TENSION OF MILK BY THE ADDITION OF SODIUM SALTS

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For a good many years some formulae used in infant feeding have specified the addition to the milk of salts, such as sodium citrate. Bosworth and VanSlyke (1) in 1914 in commenting on the earlier work of Talbot done in 1905 in which he showed normal milk to form large curd particles in the human stomach, stated, "These lumps of curd may pass practically unchanged through the entire intestinal canal, causing mechanical irritation, which often results in serious interference with the process of normal digestion. Empirical practice has shown that this abnormal curdling of milk may, to some extent, be modified or controlled by the addition of sodium citrate at the rate of 1 or 2 grains per ounce of milk." Bosworth and VanSlyke explained the failure of milk containing sodium citrate to curdle upon the addition of rennet as being due to the formation of calcium sodium paracaseinate which is quite soluble. While other methods have been employed to reduce curd tension in milk, such as high-heat treatment, base exchange, addition of enzymes, and homogenization, so far as the authors are aware no commercial operations are practiced which involve the direct addition of sodium salts, even though the work of Bosworth and VanSlyke, done 25 years ago, clearly indicated the possibilities of such a practice. Schwartz, Jones, Mack, and Vance (2) reported at the 1939 annual meeting of the American Dairy Science Association upon the use of sodium metaphosphate for the preparation of soft-curd milk.

EXPERIMENTAL

The method of measuring curd tension in this study was the one recommended by the committee on curd tension measurement (of the American Dairy Science Association) which reported at the 1938 meeting in Columbus, Ohio. This method involves the use of a pepsin hydrochloric acid coagulant. The curdometer used was one manufactured by the Submarine Signal Company. The milk was supplied by the University herd.

While it is recognized that there are several salts of sodium that might be successfully used to soften the curd of milk, this study was limited to a consideration of sodium citrate, sodium pyrophosphate and sodium hexametaphosphate.

USE OF SODIUM CITRATE

To study the use of sodium citrate as a softener of the curd of milk, varying amounts of the salt were added to raw milk which was heated to

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143° F. and held for 30 minutes. Some of the milk was then homogenized at 2500 pounds pressure. Data were secured on the curd tension values of the raw, pasteurized, and homogenized milk, the flavor of the milk and the creaming qualities of the pasteurized milk.

TABLE 1

Effect of sodium citrate upon curd tension of milk

Sodium-citrate	Curd tension		
	Raw	Pasteurized	Homogenized
per cent	*grams*	*grams*	*grams*
None	46	43	13
.10	27	30	14
.15	18	23	13
.20	0	1	3
.25	0	0	0

It is interesting to note that the curd tension of the milk to which sodium citrate had been added was higher after pasteurization. It will also be observed that up until zero curd tension was obtained the proportional decrease in curd tension caused by the sodium citrate was greater in the case of both the raw and pasteurized milk than it was in the case of the homogenized milk.

The titratable acidity of all the milks to which sodium citrate was added was only slightly affected, being reduced from 0.15 to 0.145 per cent. The flavor of the milks was made salty by the addition of sodium citrate but peculiarly the salty flavor was less noticeable in the case of the homogenized milk.

That the sodium citrate in the amounts used had no appreciable effect upon the creaming qualities of the milk to which it was added is shown by the data in table 2.

TABLE 2

Effect of sodium citrate upon creaming of pasteurized milk

Sodium citrate added	Cream volume				
	2 hrs.	5 hrs.	8 hrs.	24 hrs.	48 hrs.
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
None	15 –15	17 –16.5	17 –16	17 –16	14.5–15
.10	14 –13.5	16 –16	16.5–15	18 –17	15.5–16.5
.15	11 –12	11 –12	.....	14 –15	15.5–15.5
.20	14.5–14.5	16 –16	17 –16.5	18 –16.5	17 –16
.25	11 –12	14 –13	14 –14	15.5–15.5	15 –15.5

Upon adding 2 parts per million of copper to the pasteurized experimental milks it was found that there was less tallowy flavor developed in the milks to which the sodium citrate was added.

USE OF SODIUM PYROPHOSPHATE

Having determined that a sodium salt such as sodium citrate would produce an appreciable reduction in the curd tension of milk an attempt was then made to find a sodium salt that would produce similar effects upon the curd tension of milk with less effect upon the flavor. For this purpose sodium pyrophosphate, a salt commonly used as an emulsifier in the manufacture of process cheese, was first tried. Table 3 shows the relative effects which the same amounts of sodium citrate and sodium pyrophosphate had upon the curd tension of milk. The greater effect of the pyrophosphate is clearly shown. The effect of heat in slowing up the effect of this salt, and its proportionally lesser effect upon the curd tension of homogenized milk is further indicated by the results in table 3.

TABLE 3

*Relative effect of 0.15 per cent sodium citrate and 0.15 per cent sodium pyrophosphate upon the curd tension of milk**

Sample	Curd tension	
	Sodium citrate	Sodium pyrophosphate
	grams	*grams*
1. Salt added to pasteurized milk before heating to 143° F. for 10 minutes	27	4
2. Salt added to pasteurized milk not heated	18.5	0
3. Same as 1 except homogenized after pasteurization but before addition of salt	15.5	4
4. Same as 3 except not reheated after addition of salt	6	2.5

* Curd tension of control pasteurized—48 grams.
Curd tension of control pasteurized and homogenized—11 grams.

An additional comparison of sodium citrate and sodium pyrophosphate is given in table 4. In this experiment an attempt was made to combine the

TABLE 4

Reducing curd tension by combining the effects of high-heat treatment and the addition of a sodium salt

Heat treatment given milk	Curd tension		
	Control	0.15 per cent Na citrate	0.10 per cent $Na_4P_2O_7$
	grams	*grams*	*grams*
143° – 30 minutes	54	44	30
170° – 0 minutes	49	24	16
" – 2 "	39*	18*	14.5
" 4 "	34	8	8.5
" 6 "	30	7	3.0*
" 8 "	30	4.5	3
" 10 "	24	2.5	3

* Indicates point where cooked flavor became sufficiently strong to be noticeable.

effects of high-heat treatment and the sodium salts in producing soft-curd milk. For this purpose the milk was heated rapidly to 170° F. and a sample immediately taken and other samples taken every 2 minutes for 10 minutes. The temperature of 170° F. was arbitrarily selected as one representing the upper limit to which milk could be heated without acquiring an objectionable cooked flavor. The amounts of sodium salts used were such as to produce an appreciable effect upon the curd without too noticeable an effect upon the flavor of the milk. It is interesting to note that less cooked flavor was detectable in the milk to which the pyrophosphate was added. It would seem that where lack of cream line is not a factor, a satisfactory low-curd tension milk could be produced by heating the milk to 170° F. for 4 to 5 minutes, cooling rapidly and then adding the sodium salt.

That this treatment would not be particularly advantageous in the case of homogenized milk is shown by table 5.

TABLE 5

Reducing curd tension of homogenized milk by combining the effects of heat treatment and the addition of 0.1 sodium pyrophosphate

Treatment given milk	Time $Na_4P_2O_7$ added	Curd tension
		grams
Past., homo.,—heated 170° F., 5 min.	Not added	10.5
" " " " " "	Before homo.	7.0
" " " " " "	After homo., heating and cooling	4.0
Pasteurized at 143.5° F. and homogenized	Not added	14.0
" " " " " "	Before homo.	13.0
" " " " " "	After homo., and cooling	11.5
Pasteurized at 143.5° F.	Not added	52

USE OF HEXAMETAPHOSPHATE TO REDUCE CURD TENSION IN MILK

Previous mention has been made of the use of sodium metaphosphate by Schwartz *et al.* to produce soft-curd milk. However, since this salt hydrolyzes rapidly to form dihydrogen phosphate (NaH_2PO_4) which is rather inactive at the pH of milk, it was thought best to use the hexametaphosphate in our studies. This salt hydrolizes rather rapidly in the presence of heat to form dihydrogen phosphate. A commercial form of hexametaphosphate (Calgon) on the market as a water softener was also studied. This product contains a small amount of soda ash to reduce hydrolysis.

In the preliminary trials the hexametaphosphate was found to be more effective than pyrophosphate in reducing curd tension. As would be expected, heating the milk with the salt resulted in a higher curd tension than that obtained by adding the salt to the cold milk. Undoubtedly this was due to the hydrolysis of a certain portion of the hexametaphosphate to

form the less active dihydrogen phosphate. A comparison of the four salts studied is given in table 6.

TABLE 6

Comparison of different sodium salts in their effect upon curd tension and flavor of milk

Sample	Curd tension				Flavor (after 24 hrs.)
	Fresh	24 hrs.	48 hrs.	72 hrs.	
	grams	*grams*	*grams*	*grams*	
1. Control	51	51	50	48	Satisfactory
2. .05% Calgon	38	35	27	28	Slight slickness and burning
3. .075% "	8.5	5.5	0	0	" "
4. .10% "	0	1	0	0	" "
5. .05% hexametaphosphate	36.5	35	28	26	" "
6. .075% "	5.5	3	0	3	" "
7. .10% "	0	0	0	0	" "
8. .10% pyrophosphate	33	33	29	27	" "
9. .15% "	12	13	12	13	" "
10. .20% "	5.5	4.5	1.5	4	" "
11. .10% sodium citrate	38	40	33	31	Slightly salty
12. .15% " "	31	31	22	21	" "
13. .20% " "	12	12	3	0	" "

The titratable acidity of the milk was practically unaffected by the salts. Cream-line measurements of each sample were made periodically up to 48 hours and, with the exception of the sample containing 0.20 per cent pyrophosphate, there were no significant differences. The sample referred to was noticeably more viscous than the others and failed to show any cream line even after 48 hours.

DISCUSSION

A permanent reduction in the curd tension of milk can be obtained by the addition of sodium citrate, sodium pyrophosphate, or sodium hexametaphosphate. The amounts required will vary with the milk and the results desired. When using milk of approximately 50 grams curd tension, 0.075 per cent of the sodium hexametaphosphate, 0.15 per cent of the sodium pyrophosphate or 0.20 per cent of the sodium citrate are necessary to reduce the curd tension to 0–10 grams.

The flavor of the milk is affected to some extent by the addition of these salts. The citrate produces a salty flavor while the phosphate salts produce a slight burning sensation at the edges and tip of the tongue. All of the milks after treatment have a slight slickness of body. It was found possible to produce a superior flavored product by combining equal portions of samples 6 and 12 (table 6) suggesting that the flavor effect of the two salts might be minimized by using an amount of each salt necessary to produce in their combined effect the desired curd tension. This was done in one case by using 0.0375 per cent of the hexametaphosphate and 0.075 per cent of the sodium citrate.

A lower curd tension results when the sodium salts are added after pasteurization to the cooled milk. After adding the salt, the curd tension continues to decrease, reaching an equilibrium after about 48 hours.

Normal creaming of the milk is not affected until sufficient salt is added to change the viscosity. The titratable acidity of the milk remains practically unchanged after treatment.

The desirability of using sodium salts to lower the curd tension of milk remains to be established. Before the procedure is used commercially it should be proven by sufficient clinical evidence that milk with a curd tension lowered by the application of sodium salts has superior nutritional qualities. It also will be necessary to have official sanction from the health officials since such additions to milk ordinarily would be considered adulteration.

SUMMARY

A method has been presented for producing milk with reduced curd tension by the addition of certain sodium salts. It is indicated that there is need for further nutritional study of the product before recommending adoption of the method by the industry.

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POTENTIOMETRIC STUDIES WITH RESAZURIN AND METHYLENE BLUE IN MILK[1]

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Comparative studies with resazurin and methylene blue as indicators of the bacteriological quality of milk have been carried on in these laboratories for several years. It has been found that the resazurin "one hour" test is less reliable than the methylene blue test in that milks containing large numbers of dormant organisms frequently fail to cause any significant change in color during this period (8). Furthermore, seasonal and breed differences in pigmentation complicate color comparisons, while some workers have difficulty in distinguishing the intermediate shades of color. When, however, incubation is continued to the pink stage, there is an excellent correlation between reduction times for resazurin and methylene blue, the ratio being approximately 3:4[2] (fig. 1). Calculation of the coefficients of correlation between reduction times and Breed counts of individual cells for 369 samples of market milk yielded the following values:

Resazurin: $r = -0.711 \pm 0.017$

Methylene blue: $r = -0.651 \pm 0.020$

Consequently, this modified resazurin test, using the pink end-point, may be considered at least as accurate as the methylene blue test as an index of the bacterial content of milk at the time the test is begun.

We have been interested in discovering the reason for the significantly shorter time required for the reduction of resazurin to the pink stage. Resazurin is slightly more electropositive than methylene blue (9). However, the shape of the time-potential curve ordinarily obtained with either plain milk or milk + methylene blue is such that it is difficult to believe that the slight difference E'_o values for these two dyes is sufficient to account for the marked shortening in reduction time[3] indicated in figure 1. The only other explanation that suggests itself is that resazurin may change the shape of the time-potential curve so that the zone of reduction will be reached somewhat earlier than with milk + methylene blue or plain milk. Studies were

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[2] In these studies, the errors resulting from undisturbed creaming (3, 7, 10, 13) were minimized by inversion of tubes at regular intervals until incipient reduction was noted. The wider ratio (1:2) reported by Collins *et al.* (1) may be due to their incubating tubes undisturbed, or to end-point differences.

[3] Reduction time for resazurin represents the time taken to change to a full pink color; for methylene blue, the time required to decolorize at least 90 per cent of the column of milk.

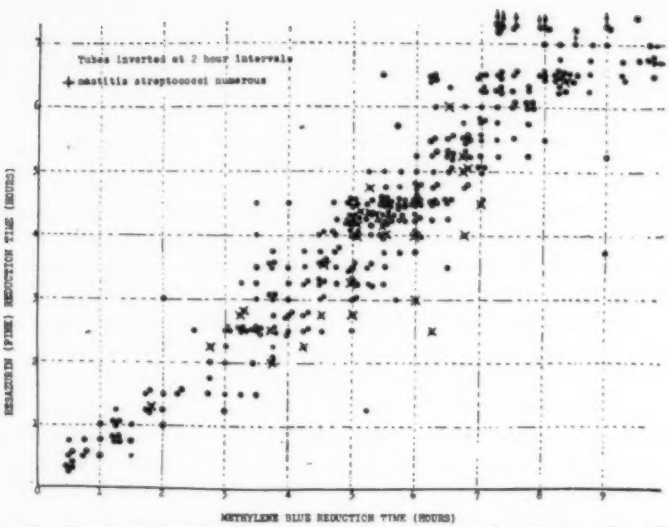
Tubes inverted at 2 hour intervals
+ mastitis streptococci numerous
RESAZURIN (PINK) REDUCTION TIME (HOURS)
METHYLENE BLUE REDUCTION TIME (HOURS)

FIG. 1. Comparative reduction times with resazurin and methylene blue for 369 market milks.

therefore undertaken to shed some light on this and other points in connection with these dye reduction tests.

EXPERIMENTAL

The electrode vessels consisted of 1″ × 8″ test tubes, closed with 3-hole rubber stoppers through which passed two spiral platinum electrodes and a tube containing saturated KCl. The latter was narrowed at the base and crimped onto asbestos fiber to prevent the rapid leakage of the solution. The top of the tube was flared to facilitate insertion of a KCl-agar bridge.[4] Six tubes were set up at one time, connections being made to a Beckman Model G vacuum tube potentiometer through a specially constructed 12-way switch.

After autoclaving while filled with distilled water, the tubes were emptied and 2.5 ml. of dye solution[5] added. Twenty-five ml. of milk were then introduced into each tube and the tubes set in a water-bath at 37° C. In general, half of the tubes were carefully inverted at hourly intervals, the remainder being disturbed as little as possible.

Although at times marked variability characterized the readings from

[4] This type of connection was devised and constructed by Mr. G. B. Landerkin, M.Sc., of this Division.

[5] Resazurin (EK 2106) to give a concentration of 1: 200,000 in milk. Methylene blue thiocyanate (Na. t1) to give a concentration of 1: 300,000 in milk. An equivalent quantity of sterile distilled water was added to control tubes.

duplicate electrodes, it was soon evident that the shape of curve for milk plus resazurin differed from that for plain milk or milk plus methylene blue. The curves shown in figures 2 and 3 are representative of those from a wide

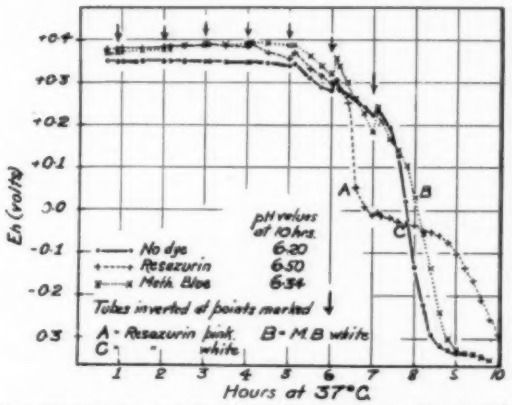

FIG. 2. Time-potential curves for milk, milk plus resazurin and milk plus methylene blue at 37° C.

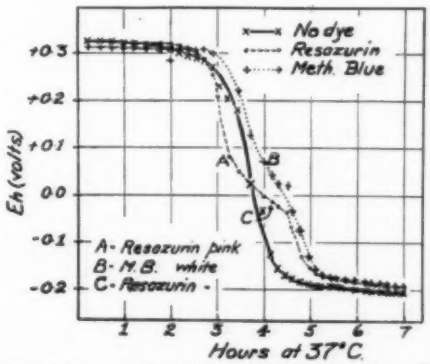

FIG. 3. Time-potential curves for milk, milk plus resazurin and milk plus methylene blue at 37° C.

variety of milks. It will be noted that the curve for milk plus resazurin shows a sharper initial drop in Eh than do those for milk plus methylene blue or plain milk. This drop is followed by a flattening of the curve, starting at a point near that at which the full pink color appears. Later the curve again declines, eventually reaching the same final Eh level as the others.

This flattening of the curve after reaching the pink stage suggests that resazurin exerts a stronger poising action than does the older dye. Time-potential curves obtained with portions of the same lot of milk containing

one-half strength, full strength and double strength resazurin are shown in figure 4. (In this graph the scale of the X axis has been doubled in order

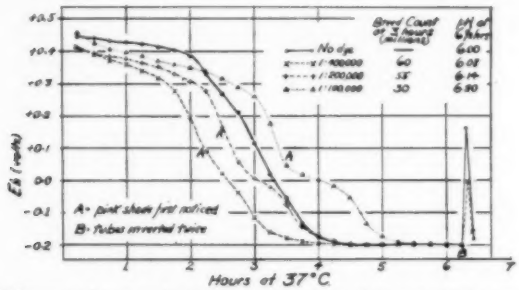

FIG. 4. Influence of resazurin concentration upon time-potential curves at 37° C.

that differences may be more readily noted. The points at which the appearance of the pink color were noted are only approximations.) These curves indicate: 1, that the degree of flattening of the curve is proportional to the dye concentration, and 2, that the double strength dye exerts a definite bacteriostatic influence. The latter is also borne out by the pH values at 6½ hours and by the Breed count of individual organisms made from a parallel set of tubes at the 3rd hour.

Turning now to the influence of inversion upon the time-potential curve (figs. 2 and 5) it will be noted that it causes little or no change during the

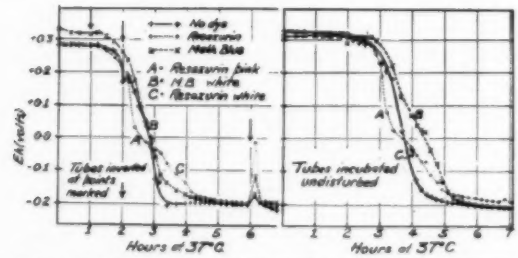

FIG. 5. Influence of mixing upon time-potential curves.

first period when the curve is approximately horizontal. As the Eh drops, however, the effect of inversion becomes more pronounced. However, the Eh changes are of a transient nature and have little influence upon the general trend of the curve. On the other hand, dispersion of the cream layer and accompanying bacteria frequently results in much earlier reduction of both resazurin and methylene blue (fig. 5). That this may be due to more active growth is indicated by the higher hydrogen ion concentration in mixed than in unmixed tubes at the end of the run (table 1). The data

TABLE 1

*Influence of hourly inversion of tubes upon final pH values**

Date	Hours incubated at 37° C.	Plain milk		Milk + meth. blue		Milk + resazurin	
		A	B	A	B	A	B
Nov. 22	9½	5.97	5.51	6.02	5.68	6.05	5.80
" 24	9¾	5.85	5.52	6.00	5.65	6.00	5.70
" 25	10½	6.00	5.81	6.21	6.00	.....	.....
Dec. 2	9½	6.18	6.00	6.28	6.10	6.31	6.18

* Determined with glass electrode.
A = Tubes incubated undisturbed.
B = Tubes inverted hourly until incipient reduction noted.

in table 1 indicate that at the concentrations employed, resazurin exerts a slightly greater bacteriostatic influence than does methylene blue. Experience with several hundred samples of market milk has failed to reveal any undesirable effect attributable to inversion of tubes at intervals of 1 to 2 hours during incubation. In passing, attention may be called to the unusually low final Eh levels reached in certain samples (figs. 2 and 6). Some of

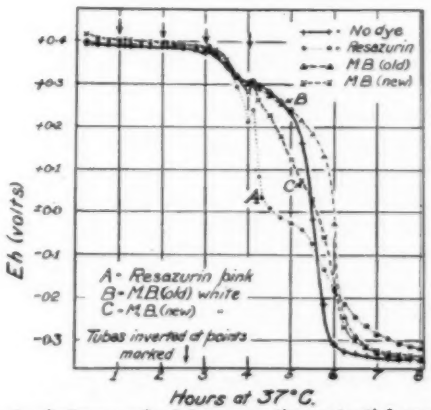

FIG. 6. Influence of mixing upon time-potential curves.

these were taken from a 3,000 lb. vat, others from the weigh-tank (individual shippers). Wilson (13) reports similar low levels in pure culture studies with *Esch. coli* and *Aerobacter aerogenes,* but we have seen none recorded for mixed milks.

It is now generally realized that certain organisms show much less active reduction of potential than do the majority of bacteria (4, 5, 13). In at least one area, milks containing numerous thermoduric organisms have failed to reduce methylene blue within 8 hours when incubated undisturbed. In view of its higher Eh range, and of the earlier downward bend of the

curve, it seemed likely that resazurin should prove useful in detecting such milks.

Through the courtesy of Dr. E. H. Parfitt, representative cultures of these thermoduric organisms have been tested out with both dyes in the ordinary reduction test as well as potentiometrically. Only a gradual decline in Eh has been observed even when the direct microscopic count at the start showed 5,000,000 individual bacteria per ml.[6] The color changes in the dye-milk mixtures are correspondingly gradual. There is usually partial reduction of methylene blue by the time resazurin reaches the pink stage. However, a trace of blue color remains in the body of the tube for some hours.[7] Data presented in table 2 suggests that if the pink stage of resazurin is taken as the end-point, this test will grade these milks somewhat more satisfactorily than will methylene blue. Since the optimum temperature for these organisms is below 37° C., growth is encouraged by preliminary incubation at 12.8° C. for 18 hours, as previously advocated (6), and the reduction time significantly shortened.

Substitution of methylene blue thiocyanate for the chloride, and increase in dye concentration to 1:300,000 have been recommended (12). The latter modification lengthens reduction time, Thornton and Sandin (12) reporting an increase of 30 minutes, Johns (7) 20 per cent and Frayer (3) 100 per cent increase. Although Thornton *et al.* (11) report reduction over the same approximate Eh range for both dye concentrations, our studies indicate that this lengthening of reduction time is due to the increase in dye concentration, as suggested by Fay and Aikin (2). Despite the obvious difficulty of determining the exact potential at which decolorization occurs, we have invariably noted visual reduction at a definitely higher level with the weaker concentration. In several instances the average value from duplicate electrodes has been almost 200 mv. more positive. The points noted in figure 6 are fairly representative of a variety of milks we have studied potentiometrically.

SUMMARY

Resazurin reduces to the pink stage in approximately three-fourths of the time required for methylene blue to decolorize. Potentiometric studies indicate that this shortening of reduction time is mainly attributable to the change in shape of the time-potential curve when resazurin is present.

Hourly inversion of tubes during incubation generally shortens the reduction time of good milks considerably. The incorporation of oxygen by this practice has little or no effect upon the time-potential curve during the early stages, and only transient effect later.

[6] This may be attributed to the slow growth rate at 37° C. When incubated at room temperature, growth is more rapid and the Eh curve shows a steeper decline.

[7] With both dyes a narrow zone of fairly intense color may remain at the surface for hours after the resazurin pink stage has been reached.

TABLE 2

Comparison of resazurin and methylene blue in evaluating milks containing weakly reducing organisms

Cult. No.	Resazurin color number* after (hours)									Reduction time (hours)		Initial Breed count individual cells per ml. (thousands)
	1	2	3	4	5	6	7	8	9	Resazurin†	Meth. blue	
1	2	6	8	8	8	8	13	20		7½	>9	175
2	14	23	24							1¼	2	29,400
4	6	10	12	16	16	16	16	16		4	9	22,600
5	2	22	24							1½	2½	4,670
8	2	16	20	24						2	3½	18,200
1	1	2	2	2	2	4	8	12	16	9	>9	2,540
2	2	4	5	6	14	18	21	23		5½	8½	2,860
4	5	9	11	12	15	17	17	17		5½	>9	9,020
5	1	2	2	2	2	8	13	18		7¼	>9	2,220
8	1	3	3	4	6	13	19	19		6¼	>9	2,960
4	†	12	15	17	18	19	21	23		3½	4½	18,600
4	8	12	16	16			20		24	3	8½	38,100

* Color numbers range from initial full color (0) to pink (16) and to white (24).
† Pink taken as end-point of reduction.

Resazurin is more useful than methylene blue in detecting milks containing large numbers of weakly reducing organisms.

The lengthening of reduction time when the concentration of methylene blue is increased from 1:700,000 to 1:300,000 appears to be attributable to a downward shift in the Eh zone at which the dye decolorizes.

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EFFECT OF LACTIC ACID ON THE HYDROLYSIS OF FAT IN CREAM BY PURE CULTURES OF LIPOLYTIC MICRO-ORGANISMS[1]

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It has been reported that the development of lactic acid in milk or cream has a restraining influence on the growth of undesirable types of micro-organisms. To study this problem, sterilized cream was inoculated at approximately the same time with a butter culture and a culture of a common lipolytic micro-organism. Lipolytic molds, yeasts and bacteria were used in these trials and included *O. lactis, Myc. lipolytica, Ach. lipolyticum, Alc. lipolyticus* and *Ps. fluorescens.* After inoculation, the lots of cream were held at 21° C. The acidity and flavor of the cream and acid number of the fat were determined after 2, 4 and 6 days.

After 6 days incubation at 21° C. (table 1) the fat in the sample inoculated only with the mold had an acid number of 40.4 while the sample inoculated with butter culture organisms as well as *O. lactis* had an acid number of 10.4. It is evident that the growth of butter culture organisms with the resultant formation of lactic acid inhibited the lipolytic activity of *O. lactis* in cream.

After 6 days incubation the sample containing only *Myc. lipolytica* had a fat acid number of 37.9 while the acid number of the fat in the sample inoculated with butter culture organisms as well as the yeast was 47.9. This indicates that the lactic organisms growing in the cream stimulated increased lipolytic action by the yeasts.

After 6 days incubation, the acid numbers of the fat of cream inoculated with cultures of lipolytic bacteria, with and without butter cultures respectively, were as follows: *Ach. lipolyticum* 2.5 and 8.3; *Alc. lipolyticus* 1.6 and 2.5 and *Ps. fluorescens* 2.5 and 4.4. All three species of lipolytic bacteria used in this study were definitely inhibited in their activity by the growth of butter culture organisms.

The question arose as to whether this inhibition was simply the result of the formation of lactic acid in the cream or whether the presence of growing butter culture organisms might have exerted some influence on the oxygen demands or other growth needs of the lipolytic organisms. In order to determine this point, lactic acid was sterilized and added to sterilized cream in such amounts that samples of the same lot of cream were obtained, varying in reaction from sweet in the check sample to very sour in the acidulated samples. These samples of cream were inoculated with various lipolytic

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[1] From a portion of a thesis presented to the Graduate School of Iowa State College in partial fulfillment of the requirements for the Ph.D. degree.

TABLE 1

Effect of the growth of butter culture organisms in cream on the growth of lipolytic organisms

Days held at 21° C.	Butter culture added			No butter culture added		
	Per cent acidity	Acid number	Flavor of cream	Per cent acidity	Acid number	Flavor of cream
			O. lactis			
2	0.86	10.9	acid, rancid	0.17	12.5	fruity
4	.81	6.5	acid, rancid	.22	24.8	rancid, fruity
6	.77	10.4	acid, rancid	.24	40.4	rancid, fruity
			Myc. lipolytica			
2	.86	2.7	rancid	.23	8.8	rancid
4	1.04	20.1	bitter, rancid	.38	20.1	rancid, bitter
6	.98	47.9	bitter, rancid	.38	37.9	rancid, bitter
			Ach. lipolyticum			
2	.76	1.3	acid, old	.26	2.4	old
4	.95	2.1	acid, old	.31	4.5	acid, rancid
6	.96	2.5	acid, old	.37	8.3	rancid
			Alc. lipolyticus			
2	.81	.5	acid	.19	.8	good
4	.87	1.0	acid	.23	2.4	old
6	.88	1.6	acid	.23	2.5	sl. rancid
			Ps. fluorescens			
2	.88	1.4	acid, unclean	.19	1.4	rancid
4	.92	2.0	acid, unclean	.27	3.5	rancid, putrid
6	.96	2.5	acid, old	.27	4.4	rancid, putrid

micro-organisms. After incubating for 6 days at 21° C., the samples were churned and the acid numbers of the fat determined. As a check on the effect of the acid on the acid number of the fat, a series of acidulated but uninoculated samples was held for the same period as the inoculated samples and the acid numbers of the fat determined.

As shown in table 2, the uninoculated cream ranged in titratable acidity from 0.18 per cent (check sample) to 2.08 per cent in the sample receiving the largest portion of added lactic acid. No differences were observed in the acid numbers of the fat of this entire series of cream samples after incubation. These data indicate that lactic acid, even in concentrations greater than normally formed in cream, did not cause an increase in the acid number of fat.

O. lactis when inoculated into sweet or moderately sour cream increased the titratable acidity appreciably probably due to the liberation of acids from the fat. As the amount of added lactic acid was increased the action of the molds on the fat decreased. The samples of cream having titratable acidities of less than approximately 0.50 per cent when inoculated, showed

increases in titartable acidity due to the growth of the mold; samples having titratable acidities over approximately 0.50 per cent when inoculated, showed decreases. In general, the lower the titratable acidity of the cream when inoculated with *O. lactis*, the higher the acid number of the fat became, due to mold growth. From the data (table 2) it may be seen however that marked increases in the acid number of the fat were observed even when the mold was inoculated into cream having titratable acidities in excess of 1.0 per cent.

TABLE 2

Effect of the addition of lactic acid on the growth of lipolytic micro-organisms in cream

Lot	Cream acidulated—not inoculated		Cream acidulated and incubated 6 days at 21° C. after inoculation with					
			O. lactis		*Myc. lipolytica*		*Ach. lipolyticum*	
	Per cent acidity	Acid number of fat	Per cent acidity	Acid number of fat	Per cent acidity	Acid number of fat	Per cent acidity	Acid number of fat
1	0.18	0.5	0.46	26.1	0.60	.....	0.41	11.3
2	.24	.7	.29	19.6	.46	.....	.41	8.9
3	.41	.5	.45	15.8	.52	27.8	.49	7.8
4	.52	.7	.45	18.4	.60	21.8	.55	7.8
5	.71	.5	.55	14.0	.74	21.4	.66	5.0
6	.97	.5	.60	8.5	.85	13.7	.88	6.4
7	1.34	.5	.86	4.7	1.12	12.8	1.36	1.0
8	2.08	.6	1.34	3.7	1.72	3.1	2.17	.9

Myc. lipolytica when inoculated into samples of cream having varying acidities due to added lactic acid apparently grew luxuriantly even in the samples having very high titratable acidities. In table 2 it may be observed that a marked increase in the acid number of the fat occurred when cream having an acidity of 2.08 per cent was inoculated with this organism. In the cream having acidities above approximately 0.70 per cent, the organisms apparently utilized the acid in their growth since decreases in titratable acidities were observed in these samples.

Ach. lipolyticum formed some acid when growing in cream and in some of the trials was able to hydrolyze fat when inoculated into cream having a titratable acidity of about 1.0 per cent (table 2) as is evidenced by increased acid numbers of the fat in these samples.

The organisms studied were types which are commonly found in cream, and it seems that it is not safe to assume the undesirable types of organisms will be controlled in cream having high titratable acidity. It can readily be seen that any of the organisms studied might cause appreciable damage to the quality of cream even though it was sour. Lactic acid in cream in quantities greatly exceeding the amount normally produced by ordinary milk souring organisms, was definitely not a factor contributing to increased acid numbers in butterfat.

CONCLUSIONS

1. *O. lactis* and all of the species of bacteria studied were inhibited somewhat by the growth of butter culture organisms in cream; *Myc. lipolytica* showed increased growth in the presence of the butter culture organisms. Lipolysis, even in high acid cream, was extensive enough with all organisms investigated to be of importance in cream quality.

2. *O. lactis*, *Myc. lipolytica* and *Ach. lipolyticum* were definitely inhibited by the addition to cream of excessive amounts of lactic acid. However, they all grew well in cream containing sufficient added lactic acid to give a titratable acidity of approximately 1.0 per cent. The first two species caused lipolysis in cream with an acidity of 2.08 per cent.

3. The addition of lactic acid to sterilized cream in amounts sufficient to increase the titratable acidity up to 2.08 per cent did not cause changes in the acid numbers of the fat after holding 6 days at 21° C.

RELATION OF VOLATILE ACIDITY OF BUTTERFAT TO RANCIDITY[1]

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In the trials reported in preceding papers the acid numbers of the fat of many samples of fresh cream were determined. Without exception the acid numbers of fresh fat were less than 1.0 and were usually between 0.5 and 0.6. Volatile acidity determinations on these samples of fat invariably yielded such low values that no dependence could be placed on them because they were usually lower than the limit of error of the titration method employed.

VOLATILE AND NON-VOLATILE ACIDITY RELATIONSHIPS IN THE FAT OF COMMERCIAL BUTTER SHOWING RANCIDITY

Among the many samples of commercial butter examined there were a number which were rancid. Acid number and volatile acidity determinations were made on the fat of these samples.

The data shown in table 1 reveal that the samples of commercial butter which were described as rancid in some degree had acid numbers on the fat ranging from 1.3 to 14.0. Samples 1, 4 and 5 were described as rancid with acid numbers of 4.8, 5.6, and 5.8, respectively, while samples 3 and 9 were described as slightly rancid and had acid numbers of 10.8 and 14.0, respectively. Sample 13 was very rancid and had an acid number of only 1.6 and sample 14 was rancid with an acid number of only 1.3. There was no correlation between the intensity of the rancid flavor and the acid number of the fat.

Samples 1 to 12, inclusive, revealed relatively little variation in the volatile-non-volatile acid relationships of the fat acidity. The percentages of the total acid that were volatile ranged from 11.4 to 16.7. Samples 13 and 14 had very low acid numbers and did not yield a sufficient quantity of volatile acidity to measure accurately by the method employed.

The data show that little or no correlation existed between rancidity and acid number of the butterfat. In the data (table 1), it may be noted that the acid number of the fat and the percentage of the total acid that was volatile was not related directly to the intensity of the rancid odor. While it is rather unsatisfactory to determine the degree of rancidity by organoleptic tests, no other method exists which will detect the defect with equal reliability. Probably the agent responsible for the hydrolysis is an important factor in determining the degree of rancidity that will accompany a certain acid number on the fat. For example, in some experimental trials,

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[1] From a portion of a thesis presented to the Graduate School of Iowa State College in partial fulfillment of the requirements for the Ph.D. degree.

TABLE 1

Volatile and non-volatile acid relationships in the fat of commercial butter showing rancidity

Sample	Flavor	Acid number	Volatile acidity*	Per cent of total acid in the fat	
				Volatile	Non-volatile
1	rancid	4.8	0.8	16.7	83.3
2	very rancid	6.2	1.0	16.1	83.9
3	sl. rancid	10.8	1.55	14.3	85.7
4	rancid	5.6	.65	11.6	88.4
5	rancid	5.8	.95	16.4	83.6
6	sl. rancid	2.8	.45	16.1	83.9
7	sl. rancid	3.2	.45	14.1	85.9
8	rancid	2.4	.4	16.7	83.3
9	sl. rancid	14.0	1.9	13.6	86.4
10	rancid	4.8	.55	11.4	88.6
11	rancid	7.6	1.15	15.1	84.9
12	sl. rancid	5.2	.75	14.4	85.6
13	very rancid	1.6	**	.....	.....
14	rancid	1.3	**	.....	.....

* The volatile acidity values represent the milliliters of N/10 sodium hydroxide required to neutralize the acid in 200 ml. of distillate when 10 gm. of fat were steam distilled.
** Quantity too small to measure accurately.

samples of butterfat on which *O. lactis* had acted showed relatively high acid numbers with no indication of rancid odor. It is possible, as has been suggested by Orla-Jensen (6), that certain lipolytic molds are able to consume the volatile acids as rapidly as they are liberated from the fat yielding a fat with a relatively high acid number and yet showing no signs of rancidity.

Hammer (4), Hunziker (5) and others have stated that the odor of rancid butter is due to the presence of some of the lower fatty acids particularly, butyric, caproic and caprylic. Grossfeld and Battay (3) reported that one part of butyric acid in 12,500 parts of a medium could be detected by sense of smell. Stark and Scheib (7) believed that amounts of butyric acid in rancid butter may be so small that though they can be detected in butter by tasting and smelling, they cannot be measured by ordinary chemical means. Since the acid numbers of some of the samples of good butter, as shown in a previous article, were very high and the acid numbers of some of the rancid samples shown in table 1 were low the agencies responsible for rancidity must have exerted a selective action on certain of the glycerides of the fatty acids. Only the higher acids must have accumulated in the good butter showing a high acid number on the fat. Conversely, in the rancid samples having very low acid numbers, a relatively large percentage of the total acid accumulated must have been volatile. From the data presented it is evident that no definite relationship existed between the quantity of acid liberated from the fat and the degree of rancidity present.

The relationship between the volatile and non-volatile acidities of the fat

of samples 1 to 12 of the rancid butter were comparatively uniform regardless of the acid numbers of the fat which ranged from 2.4 to 14.0. There was no relationship between the degree of rancidity and the volatile acidity, that is, in general the slightly rancid samples had the same volatile-non-volatile acid relationship as did the rancid and very rancid samples.

In conclusion, the percentages of the total acid in the fat that were volatile varied only slightly in the samples of rancid butter studied. There was no correlation between the percentages of the total acid in the fat that were volatile and the degree of rancidity.

EFFECT OF THE GROWTH OF VARIOUS LIPOLYTIC MICRO-ORGANISMS ON THE PERCENTAGES OF TOTAL ACID OF FAT THAT ARE VOLATILE AND NON-VOLATILE

During a study of many cases of rancidity in experimental butter caused by the growth of micro-organisms, an excellent opportunity was afforded to obtain information concerning the relationship between the volatile and non-volatile acidity in the fat of rancid butter. Portions of sterilized sweet cream were inoculated with the organisms to be studied. The cream was then incubated at 13° or 21° C. for 6 days. The acid numbers and the volatile acidities of the fat were determined in the usual manner and the percentages of total acid that were volatile were calculated; the percentages of the total acid that were non-volatile were obtained by difference. The four trials with each organism were purposely not run simultaneously and because of the fact that cultures of different ages were used and different incubation temperatures employed, the degree of fat hydrolysis in the trials was not uniform. The object was to determine whether the organisms would hydrolyze the fat into the same volatile-non-volatile acid relationship under varying conditions of growth.

The average percentage of the acid that was volatile in the four trials with *O. lactis* was 1.9 (table 2). In other trials not reported, slightly higher values were obtained but none of them was over 5.0 per cent. With *Myc. lipolytica* the percentage of total acid that was volatile was higher than with *O. lactis*, the average being 8.4. All the species of bacteria studied gave relatively high volatile acid values and were uniform in their volatile-non-volatile acid relationship. Four trials each with *Ps. fluorescens*, *Ach. lipolyticum*, and *Alc. lipolyticus* gave averages of 14.7, 11.5 and 11.2 per cent, respectively.

There was a relatively close relationship between the volatile and non-volatile acid values in all the trials with an organism, regardless of age of culture used for inoculating the cream, incubation temperature or degree of hydrolysis as shown by the acid numbers. The different organisms varied considerably in the degree of hydrolysis produced, as well as in the percentages of the total acids that were volatile and non-volatile. The *O. lactis* culture, as has previously been shown, was actively lipolytic but the volatile

TABLE 2

Volatile and non-volatile acidity relationships in butterfat produced by the growth of pure cultures of lipolytic micro-organisms in cream. Sterilized cream inoculated and held 6 days

Trial	Temperature of incubation	Acid number of fat	Volatile acidity*	Per cent of total acid in the fat	
				Volatile	Non-volatile
			O. lactis		
1	13° C.	18.6	0.45	2.4	97.6
2	13°	23.8	.5	2.1	97.9
3	21°	32.8	.6	1.8	98.2
4	21°	26.4	.4	1.5	98.5
Average	.....	.....	.....	1.9	
			Myc. lipolytica		
1	13°	9.9	.9	9.1	90.9
2	13°	12.0	1.0	8.3	91.7
3	21°	21.0	1.7	8.1	91.9
4	21°	16.8	1.4	8.3	91.7
Average	.....	.....	.....	8.4	
			Ps. fluorescens		
1	13°	3.7	.7	18.9	81.1
2	13°	5.8	.8	13.8	86.2
3	21°	7.6	1.0	13.1	86.9
4	21°	6.5	.85	13.1	86.9
Average	.....	.....	.....	14.7	
			Ach. lipolyticum		
1	13°	2.8	.35	12.5	87.5
2	13°	4.7	.5	10.6	89.4
3	21°	7.1	.8	11.5	88.5
4	21°	8.3	.95	11.4	88.6
Average	.....	.....	.....	11.5	
			Alc. lipolyticus		
1	13°	5.4	.65	12.0	88.0
2	13°	8.7	1.0	11.5	88.5
3	21°	14.9	1.6	10.7	89.3
4	21°	12.8	1.4	10.9	89.1
Average	.....	.....	.....	11.2	

* See table 1.

acidity of the fat on which it had acted was somewhat low as compared with all other organisms studied. This observation confirms the belief of Orla-Jensen (6) who suggested that the organism utilized in its metabolism, the volatile fatty acids liberated by its growth.

For reasons previously cited the degree of hydrolysis caused by the same organism in different trials, as determined by acid numbers, varied somewhat. With a few exceptions, all the trials with an organism gave volatile acid values that were quite uniform, regardless of the degree of hydrolysis. In the case of *O. lactis* the acid numbers ranged from 18.6 to 32.8 but the vola-

tile acid percentages varied only from 1.5 to 2.4. With *Myc. lipolytica* the acid numbers ranged from 9.9 to 21.0 and the volatile acid percentages from 8.1 to 9.1. With *Ach. lipolyticum* the acid numbers varied from 2.8 to 8.3 and the volatile acid percentages from 10.6 to 12.5; other bacterial species produced volatile acid values similar to those produced by *Ach. lipolyticum*. The organisms studied varied considerably in the relative percentages of the total acid liberated that were volatile and non-volatile. In all trials with the same organism however this relationship was comparatively uniform.

One organism may have attacked the fat of cream in a somewhat different manner than another, or at least the end products of the metabolic processes were different. With *O. lactis* only a relatively small percentage of the total acid was volatile after the mold had grown while with all bacterial species studied a comparatively large percentage remained after growth. With all the bacterial species studied about the same percentages of the total acid produced from the fat were volatile which in all cases were considerably higher than those produced by either *Myc. lipolytica* or *O. lactis*.

ABILITY OF VARIOUS LIPOLYTIC ORGANISMS TO UTILIZE SALTS OF THE LOWER FATTY ACIDS AS THE SOLE SOURCE OF CARBON

Both experimental and commercial rancid butter showed some variation in the volatile-non-volatile acid relationships of the fat. Various workers have suggested that certain micro-organisms utilize some of the lower volatile fatty acids in their growth. In order to determine whether the organisms used in the previously reported experiments could grow in media in which a sodium or calcium salt of a single volatile fatty acid comprised the sole source of carbon, a series of such media were prepared following the general formula of Ayres, *et al.* (1). These media had the following composition:

sodium ammonium phosphate	2.0 gm.
potassium chloride	.1 gm.
salt of fatty acid	5.0 gm.
distilled water	1000 ml.

The salts used were sodium and calcium butyrate, calcium caproate and calcium caprylate. Forty ml. portions of each medium were placed in glass containers with screw caps and sterilized in the autoclave. The media were then inoculated with micro-organisms to be studied and incubated at 21° C. After 7 days and also after 14 days of incubation, a complete series of the inoculated media were treated as follows:

The contents of each bottle were placed in a Kjeldahl flask containing 225 ml. of distilled water. Five ml. of N/1 sulphuric acid were added to each flask to free any remaining fatty acid from the salt. The flasks were then placed on the distilling apparatus and heated until 200 ml. of distillate were obtained. These distillates were titrated against N/10 sodium hydrox-

ide, using phenolphthalein as an indicator. Handled in an identical manner, uninoculated 40 ml. portions of each medium served as checks. It was assumed for comparative purposes that any decrease in the volatile acid obtained from a medium after growth of an organism, compared with the check, was due to utilization of the acid by the growing organisms.

There was no apparent uniformity in the ability of different organisms to utilize the fatty acids (table 3). *O. lactis* grew luxuriantly in the media

TABLE 3

Ability of certain lipolytic micro-organisms to utilize the salts of the lower fatty acids as the sole source of carbon

Organism	Sodium butyrate		Calcium butyrate		Calcium caproate		Calcium caprylate	
	Days of incubation at 21° C.							
	7	14	7	14	7	14	7	14
O. lactis	3.4	1.2	3.7	0.6	6.9	6.8	1.1	0.9
Myc. lipolytica	12.1	5.0	11.3	11.0	5.2	4.8	1.3	.3
Ps. fluorescens	12.5	12.3	13.0	13.0	4.7	3.6	2.0	1.7
Ach. lipolyticum	16.2	16.4	17.0	17.1	7.2	7.2	2.2	1.9
Alc. lipolyticus	12.5	9.4	10.2	8.3	7.2	7.2	2.2	1.5
Check (no inoculation)	16.1	16.2	17.5	17.1	7.2	7.2	2.9	2.9

The values represent the milliliters of N/10 sodium hydroxide required to neutralize the acid in 40 ml. of medium. The difference between the values given for an organism and the check sample (no inoculation) on the same medium represents the milliliters of N/10 acid utilized by the growing organism.

containing sodium and calcium butyrate and lowered the volatile acid obtainable from the sodium butyrate medium from 16.1 ml. (check) to 3.4 ml. after 7 days and to 1.2 ml. after 14 days. Almost complete disappearance of the butyric acid may be noted. Similar reductions were shown in the calcium butyrate and calcium caprylate media. In the calcium caproate medium some growth was evident but it was not nearly so luxuriant as in the other media. The data further substantiate earlier suggestions that *O. lactis* is able to utilize volatile fatty acids.

Myc. lipolytica grew in all the media but grew less luxuriantly than *O. lactis* in the medium containing calcium butyrate, as determined by the titration values after 14 days, the value for *O. lactis* being 0.6 ml. and for *Myc. lipolytica* 11.0 ml. This organism showed greater growth after 14 days in the media containing the calcium salts of caproic and caprylic acids than did *O. lactis*. *Ps. fluorescens* utilized all of the salts to some extent; *Ach. lipolyticum* did not show appreciable growth in any of the media and consequently utilized very little of the fatty acids. *Alc. lipolyticum* utilized sodium and calcium butyrate but was unable to utilize the calcium salts of caproic and caprylic acids to any extent.

The organisms studied varied greatly in ability to use the salts of the lower fatty acids as their sole source of carbon. The fact was established,

however, that some of the organisms studied were definitely able to destroy, by their growth, certain of the volatile fatty acids. Coolhass (2) showed that certain bacteria were able to ferment a large number of fatty acid salts. It is possible also that certain organisms may be able to act on the higher fatty acids in such a manner as to split off acetic acid causing increased titration values. It is therefore evident that the acid number of a fat is not an exact index of the degree of hydrolysis of the fat. Another point to consider is that even though certain organisms utilized the lower fatty acids in synthetic media in which the fatty acids were the only source of carbon, this does not necessarily prove that they would utilize them when growing in cream or butter. Under different circumstances they might obtain their carbon from a more readily available source and leave the fats unhydrolyzed. These results establish the possibility of the utilization of the lower fatty acids by certain micro-organisms growing in cream.

SUMMARY AND CONCLUSIONS

1. In samples of commercial unsalted butter showing widely varying degrees of rancidity, the percentages of the total acid in the fat that were volatile varied only slightly; there was no close correlation between the percentages of the total acid in the fat that were volatile and the degree of rancidity.

2. The different organisms studied varied considerably in the percentages of the total fat acid that were volatile and non-volatile. The average percentage of the total acid of the fat that was volatile in the trials with *O. lactis* was 1.9; with *Myc. lipolytica*, 8.4; with *Ps. fluorescens*, 14.7; with *Ach. lipolyticum*, 11.5 and with *Alc. lipolyticus*, 11.2.

3. There was a relatively close relationship between the volatile and non-volatile acid values on the fat in all the trials with each organism, regardless of the age of the culture used for inoculating the cream, the incubation temperature or the degree of fat hydrolysis.

4. Certain lipolytic organisms grew well in media in which a sodium or calcium salt of butyric, caproic or caprylic acid was the sole source of carbon; others grew little or not at all in these media.

5. *O. lactis* grew more luxuriantly in all of the synthetic media than any of the other organisms investigated.

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HIGH-TEMPERATURE (STEAM-INJECTION) PASTEURIZATION OF CREAM FOR BUTTERMAKING[1]

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Pasteurizers especially designed to remove certain feed flavors from cream have been introduced recently by several manufacturers. All employ direct injection of steam into the cream for heating. By mixing the steam and cream under pressures above atmospheric it is possible to heat the cream to temperatures considerably above the normal boiling point. Rapid cooling is secured by evaporation brought about by drawing the heated cream into a chamber maintained below atmospheric pressure. Constituents of the cream volatile at temperatures below the boiling point of the cream are thus removed at least in part.

Although claims have been made for equipment of this type relative to the destruction of micro-organisms, no published experimental evidence is available. McDowall (1) reported a considerably higher fat content in the buttermilk resulting from churning cream pasteurized in the Vacreator (a steam-injection, high-temperature pasteurizer) than in the buttermilk from cream pasteurized in the ordinary flash pasteurizer.

The work herein reported was undertaken to compare the results secured using one type of high-temperature, steam-injection pasteurizer with those using the standard vat pasteurizer. Factors studied included efficiency in bacterial destruction, effect on fat losses in the buttermilk, and flavor and keeping qualities of the butter.

DESCRIPTION OF EQUIPMENT

A small, high-temperature, steam-injection pasteurizer built by the C. E. Rogers Company of Detroit, Michigan, of the same design as commercial equipment sold by the same company was made available to the Dairy Division of the University of Minnesota. This equipment was used for the work concerning the fat content of the buttermilk. Due to the ease with which it could be sterilized, the apparatus described by Coulter and Combs (2) was used for the work on bacterial destruction and flavor and keeping quality of the butter. This equipment was designed to operate on the same principle as the Rogers pasteurizer. As shown in figure 1, it consists essentially of a glass tube connected at one end by means of rubber tubing with

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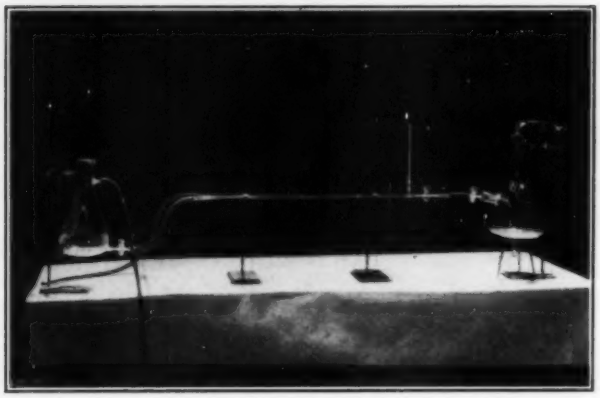

FIG. 1. Laboratory apparatus used for high temperature pasteurization.

sources of cream and steam both under 50 to 60 pounds pressure, and at the other end by the same means to a flask upon which a partial vacuum can be drawn.

The glass tube is 3 feet long and is made of standard 12-mm. O. D. Pyrex tubing. The cream and steam enter the tube through the arms of a Y. Tubes drawn to a 3-mm. orifice are fitted into the arms of the Y in order to cause the cream and steam to meet as sprays. The rate of passage of the heated cream from the tube is regulated by a stopcock placed near the end opposite that at which the steam and cream enter. A thermometer is inserted into the tube by means of a ground glass connection and is held in place by spring clips. Leaving the tube the cream enters a 6-liter Erlenmeyer flask through an opening about half way up the side of the flask. The vapors are drawn off and passed through a surface condenser and a partial vacuum maintained by means of a water aspirator.

In operation steam under pressure is admitted to the tube and after flow is established the outflow is restricted by the stopcock until a temperature of at least 265° F. is reached. Cream under pressure is then admitted to the tube at such a rate as to maintain the temperature at 260° F.

EXPERIMENTAL

Lots of both sweet and neutralized sour cream were divided into two parts. One was pasteurized at 260° F. flash by the high-temperature, steam-injection method, and the other by the standard vat process at 160° F. for 30 minutes. Other factors were held as uniform as possible.

The efficiency of pasteurization. Many of the bacteria which survive the normal pasteurization process are proteolytic in nature. These organisms are not known to be an important factor in the keeping quality of butter.

However, it is reasonable to believe that insofar as deterioration of butter due to bacterial causes is concerned, a more complete elimination of bacteria than is possible with the methods of pasteurization now in vogue is desirable. The data secured in comparing the efficiency of the vat and high-temperature, steam-injection methods of pasteurization in bacterial destruction are shown in table 1. Plating one ml. of cream direct in the standard plate

TABLE 1

Efficiency of high-temperature steam-injection and vat pasteurization as indicated by bacterial count using standard plate method

Sample No.	Pasteurization temperature	Before pasteurization	After pasteurization	Percentage killed
Lot I—Sweet cream butter		*per ml.*	*per ml.*	
1	260° F.	253,000	0	100
2	260° F.	253,000	0	100
3	260° F.	253,000	0	100
4	260° F.	253,000	1 on (D)*	100–
5	260° F.	253,000	1 on (D)*	100–
6	260° F.	253,000	0	100
7	160° F., 30 min.	253,000	19	99.99+
8	160° F., 30 min.	253,000	20	99.99+
Lot II—Sweet cream butter				
9	260° F.	92,000	0	100
10	260° F.	92,000	0	100
11	260° F.	92,000	0	100
12	260° F.	92,000	0	100
13	160° F., 30 min.	92,000	8,500	90.8
14	160° F., 30 min.	92,000	11,200	87.9+
Lot III—Sour cream butter				
1B	260° F.	231,000,000	0	100
2B	260° F.	231,000,000	0	100
3B	260° F.	231,000,000	0	100
4B	260° F.	231,000,000	0	100
5B	260° F.	231,000,000	0	100
6B	260° F.	231,000,000	0	100
7B	160° F., 30 min.	231,000,000	8,500	99.99+
8B	160° F., 30 min.	231,000,000	7,800	99.99+
Lot IV—Sour cream butter				
9B	260° F.	22,400,000	0	100
10B	260° F.	22,400,000	0	100
11B	260° F.	22,400,000	0	100
12B	260° F.	22,400,000	0	100
13B	160° F., 30 min.	22,400,000	410	99.99+
14B	160° F., 30 min.	22,400,000	350	99.99+

* (D)—plated 1 cc. direct.

count, no bacterial growth whatsoever was secured in 18 of the 20 trials with cream pasteurized at 260° F. flash and in the other trials only one colony developed. The results demonstrate a marked superiority in bacterial destruction by this method of pasteurization. Similar results should be secured by pasteurizing cream at the same temperature in commercial equipment: however, cream with a higher bacterial count may be expected due to

contamination following heating from contact with imperfectly sterilized equipment.

Flavor and keeping quality of the butter. The cream was churned in sterile, Dazey type, glass churns. The butter granules were washed with sterile water and the butter worked by hand under conditions as nearly aseptic as possible. One-half the churnings were salted to contain about 2 per cent salt and the others left unsalted. The butters were scored while fresh and after storage at 40° F. for 2, 8 and 12 weeks. Samples were taken for a standard plate count just prior to each scoring.

The average scores of the butters made from each lot of sweet cream are shown in table 2, and from each lot of neutralized sour cream in table 3.

TABLE 2

Average scores of butter made from sweet cream pasteurized at 160° F. for 30 minutes and at 260° F. flash

Lot No.	Pasteurization temperature	Score of butter			
		Fresh	2 weeks	1 month	3 months
I					
Unsalted	260° F.	92	93	93	..... *
Unsalted	160° F., 30 min.	92	93	93	..... *
Salted	260° F.	92	93	93	90
Salted	160° F., 30 min.	92	93	93	90
II					
Unsalted	260° F.	92.25	91	92.25	92
Unsalted	160° F., 30 min.	93	*	*	..... *
Salted	260° F.	93	93	93	90
Salted	160° F., 30 min.	92.5	93	93	90

* Surface taint—no scores.

TABLE 3

Average scores of butter made from neutralized sour cream pasteurized at 160° F. for 30 minutes and at 260° F. flash

Lot No.	Pasteurization temperature	Score of butter			
		Fresh	2 weeks	1 month	3 months
I					
Unsalted	260° F.	90.67	91.5	90.0	89.0
Unsalted	160° F., 30 min.	90.0	90.5	90.0	88.0
Salted	260° F.	91.0	91.18	90.0	88.0
Salted	160° F., 30 min.	90.0	91.0	89.0	87.0
II					
Unsalted	260° F.	90.75	91.0	90.0	88.0
Unsalted	160° F., 30 min.	91.0	91.0	90.0	86.0
Salted	260° F.	90.5	91.0	89.5	88.0
Salted	160° F., 30 min.	90.5	90.0	89.0	86.0

The method of pasteurization did not definitely affect either the initial score or the keeping quality of the butter. The butter made from the cream

pasteurized by the high-temperature, steam-injection method at 260° F. was uniformly criticized as having a slight cooked or custard-like flavor when fresh. This flavor was less apparent in the butter after storage. The butter judges were of the opinion that there was a less marked neutralizer and old cream flavor in the butters made from the neutralized sour cream pasteurized at 260° F. flash. This would be expected if any of the constituents responsible for these flavors are volatile at a temperature below the boiling point of the cream, since the cream boils violently when released into the vacuum chamber of the high-temperature, steam-injection pasteurization equipment. Desirable flavors if volatile would also be partially removed. This factor was not demonstrated to be of importance in this work.

The bacterial counts of the fresh and stored butters are shown in tables 4 to 7. Despite a lower initial count in the unsalted butters made from the

TABLE 4

Bacterial count by standard plate method of fresh and stored unsalted butter made from sweet cream pasteurized at 260° F. flash, and at 160° F. for 30 minutes

Churning No.	Pasteurization temperature	Bacterial count of butter			
		Fresh	2 weeks	8 weeks	12 weeks
		per ml.	*per ml.*	*per ml.*	*per ml.*
1	260° F.	0	192	3,900	25,600,000
5	260° F.	1	T.N.T.C.*	T.N.T.C.*	21,600,000
6	260° F.	2	0	2,500	4,600,000
9	260° F.	0	180	1,680	3,500,000
10	260° F.	0	4,200	280	290,000
7	160° F., 30 min.	9	1,560	T.N.T.C.*	46,000,000
13	160° F., 30 min.	350	T.N.T.C.*	680,000	14,800,000

* T.N.T.C.—Too numerous to count on highest dilution plated.

TABLE 5

Bacterial count by standard plate method of fresh and stored unsalted butter made from neutralized sour cream pasteurized at 260° F. flash and at 160° F. for 30 minutes

Churning No.	Pasteurization temperature	Bacterial count of butter			
		Fresh	Stored at 40° F. for		
			2 weeks	8 weeks	12 weeks
		per ml.	*per ml.*	*per ml.*	*per ml.*
1B	260° F.	0	2,410	175,000	98,000
2B	260° F.	0	400	89,000	176,000
3B	260° F.	0	80	2	25,000
9B	260° F.	1	30,000	310,000	1,600,000
10B	260° F.	0	20	0	1,000
7B	160° F., 30 min.	1,570	300,000	239,000	1,400,000
13B	160° F., 30 min.	50	50	610	Lost

cream pasteurized at 260° F. flash, the counts after storage were not significantly different than in the unsalted butters made from the vat-pasteurized cream. Bacteria multiply so rapidly in unripened unsalted butter when

TABLE 6

Bacterial count by standard plate method of fresh and stored salted butter made from sweet cream pasteurized at 260° F. flash and at 160° F. for 30 minutes

Churning No.	Pasteurization temperature	Bacterial count of butter			
		Fresh	Stored at 40° F. for		
			2 weeks	8 weeks	12 weeks
		per ml.	*per ml.*	*per ml.*	*per ml.*
2	260° F.	1	0	2	200
3	260° F.	1	6	1	100
4	260° F.	0	4	0	100
11	260° F.	0	10	21	0
12	260° F.	11	10	19	5
8	160° F., 30 min.	6	310	2	1,400
14	160° F., 30 min.	260	27,200	490,000	30,000

stored at 40° F. that a high count after storage is to be expected even with originally very low count butter. Even though cream can be rendered essentially sterile by high-temperature, steam-injection pasteurization, elimination of bacterial deterioration of unripened unsalted butter made from such cream cannot be expected unless contamination after pasteurization can be prevented.

The initial count of the salted butters churned from the cream flash-pasteurized at 260° F. also was lower than that of the butters from the vat-pasteurized cream. The inhibiting effect of salt on bacterial growth was evident as in no case was there an important increase in count during storage. Consequently, the lower initial count of the butters from the cream pasteurized at 260° F. although doubtless desirable, was not in this work a factor in the deterioration of the butter. Although the results might have been different if highly salt tolerant micro-organisms had been present, they are indicative at least of what might be expected under commercial conditions.

TABLE 7

Bacterial count by standard plate method of fresh and stored salted butter made from neutralized sour cream pasteurized at 260° F. flash and at 160° F. for 30 minutes

Pasteurization temperature	Bacterial count of butter			
	Fresh	Stored at 40° F. for		
		2 weeks	8 weeks	12 weeks
	per ml.	*per ml.*	*per ml.*	*per ml.*
260° F.	1	4	10	2
260° F.	1	1	0	0
260° F.	0	1	0	0
260° F.	0	10	6	4
260° F.	0	20	1	2
160° F., 30 min.	1,450	290	260	34
160° F., 30 min.	62	90	35	23

Fat losses in buttermilk. Sweet cream and neutralized sour cream were pasteurized at 260° F. flash, using the small Rogers high-temperature, steam-injection pasteurizer, and other portions of the same cream at 160° F. for 30 minutes by the vat method. After pasteurization the cream in each case was cooled, held over night and churned at 50° F. in a 64-gallon capacity Disbrow churn. The percentage of fat churned which was lost in the buttermilk from the various churnings based on the Normal Butyl Alcohol and Mojonnier tests is shown in table 8. In every case the fat lost in the butter-

TABLE 8

Per cent of fat lost in buttermilk churned from sweet and sour cream pasteurized at 160° F. for 30 minutes and at 260° F. flash

Lot No.	Pasteurization temperature	Fat test of buttermilk		Per cent* of fat churned lost in buttermilk	
		Butyl Al. test	Mojonnier test	Butyl Al. test	Mojonnier test
		per cent	*per cent*	*per cent*	*per cent*
Lot I Sour cream	160° F., 30 min.	0.46	0.64	0.93	1.30
	260° F.— I	0.84	0.88	1.95	2.04
	260° F.—II	1.02	1.16	2.37	2.70
Lot II Sour cream	160° F., 30 min.	0.9	0.92	1.65	1.69
	260° F.— I	1.5	1.55	3.14	3.24
	260° F.—II	1.6	1.69	3.35	3.54
Lot III Sour cream	160° F., 30 min.	0.5	0.59	0.91	1.06
	260° F.— I	1.0	0.96	2.29	2.20
	260° F.—II	1.1	1.00	2.59	2.29
Lot IV Sweet cream	160° F., 30 min.	0.6	0.63	0.78	0.82
	260° F.— I	1.7	1.61	2.32	2.19
	260° F.—II	1.6	1.57	2.18	2.14
Lot V Sweet cream	160° F., 30 min.	0.5	0.54	0.94	1.01
	260° F.— I	1.5	1.33	4.57	4.06
	260° F.—II	1.6	1.44	4.87	4.39

* In these calculations the weight of buttermilk was assumed as the weight of cream minus 1.2 times the weight of fat.

milk from the cream vat-pasteurized at 160° F. was much lower than that from the cream flash-pasteurized at 260° F. The difference in actual fat loss was greater than the fat percentages indicate since there was some dilution of the high-temperature, steam-injection pasteurized cream with condensed steam. A part of the water added to the cream in the form of steam is removed by evaporation in the vacuum chamber. Theoretically if the cream is cooled in the vacuum chamber to the same temperature at which it entered the pasteurizer there would be no dilution. Due to heat loss, however, some dilution occurs unless the cream is heated in the vacuum chamber to effect further evaporation. The dilution which occurred in the present work was greater than would result with large commercial equipment and based on the reduction in fat percentage varied from 5 to 15 per cent except for one churning in which the dilution was 26 per cent and another in which

it was 62 per cent. The fat loss percentages shown in table 8 have been corrected to compensate for the dilution. It is evident from the data presented that higher fat losses in the buttermilk may be expected using the high-temperature, steam-injection type of pasteurizer. McDowall (1) has reported similar results with the Vacreator. McDowall attributed the increased fat loss with cream pasteurized in the Vacreator to decrease in the size of the fat globules. Since the steam and cream are mixed under high pressure and since the flow of the heated cream is retarded by a valve in order to build up the pressure, some reduction in the size of the fat globules is to be expected. Whether this is the sole factor involved in the greater loss of fat in churning cream so pasteurized remains to be proven.

There is some evidence that the fat globule "membrane" is altered to some extent by the high heat treatment to which the cream is subjected in the steam-injection, high-temperature pasteurizer. Buttermilk from the cream so pasteurized was observed to be much whiter in appearance than normal buttermilk. Tarassuk (3) found that the brownish tint of normal buttermilk was completely and irreversibly destroyed by heating the buttermilk to a temperature above 180–185° F. Palmer and Tarassuk (4) have pointed out that the brownish tint of natural churned buttermilk is associated with the fat globule "membrane." Tarassuk (3) presented some evidence which indicates that the constituent of the "membrane" responsible for the brownish tint of churned buttermilk is associated with the protein of the fat globule "membrane."

There is no proof nor any intention to suggest that high heat treatment of cream influences its churnability, but if, as appears to be the case, pasteurization at 260° F. in the high-temperature, steam-injection pasteurizer affects the fat globule "membrane," the possibility that the churnability of the cream is influenced thereby merits consideration.

CONCLUSIONS

1. Cream which was essentially sterile as determined by the standard plate count was secured from either sweet or neutralized sour cream by pasteurization at 260° F., using a laboratory model high-temperature, steam-injection pasteurizer.

2. Salted and unsalted butter churned from such cream although having a slight cooked flavor while fresh received the same flavor score as butter churned from different lots of the same cream vat-pasteurized at 160° F.

3. Despite lower initial bacterial counts neither salted nor unsalted butter churned from cream pasteurized at 260° F. in the high-temperature, steam-injection pasteurizer kept better in storage at 40° F. than butter from different lots of the same cream vat-pasteurized at 160° F.

4. The fat lost in the buttermilk was much greater in churning cream flash-pasteurized at 260° F. in a small commercial high-temperature, steam-

injection type pasteurizer, than in churning different lots of the same cream vat-pasteurized at 160° F. for 30 minutes.

The authors wish to express thanks to the C. E. Rogers Company of Detroit, Michigan, for the loan of specially designed equipment used in these experiments.

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OFFICIAL FLAVOR CRITICISMS OF DAIRY PRODUCTS JUDGED IN THE NATIONAL CONTEST

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This analysis of the official flavor judgments of the samples of butter, cheese, milk and ice cream scored in the Students' National Contest in the Judging of Dairy Products is made: 1, that coaches may appreciate more fully the flavors recurring most frequently in the various dairy products; 2, that they may realize the association of or combination of flavors encountered; 3, that they may have an understanding of the relationship between the flavor quality of the product and the number of criticisms made, and 4, that the student may gain encouragement from the fact that although many specific off flavors are possible in dairy products, relatively few are actually encountered in the judging of normal salable dairy products. The period of the study extends from 1927, when ice cream was first introduced into the National Contest, to 1938, inclusive. During these twelve years, seven samples each of butter, cheese, milk and ice cream were judged per year.

Prior to 1930, two or more official judges were selected to place scores and criticisms on each product. Their services were not continued from year to year, although one judge may have served more than one year. Beginning in 1930, with few exceptions, the official criticisms have been placed on the samples by a selected judge for each product, and, beginning in 1932, two "coach" judges have assisted. If the three failed to agree in their judgment of the sample, that sample w s not used in the contest. As noted in "History and Development of the Students' National Contest in the Judging of Dairy Products" (1) the official judges have been retained in the same capacity each year, but the same "coach" judges have not been used continuously from year to year. Consequently the flavor criticisms placed on the samples should represent as reliable a composite judgment as it is possible to obtain. However, it must be borne in mind that in many cases specific flavor samples were selected for use in the contest and therefore the percentage distributions reported herein may not apply to commercial products as a whole.

BUTTER

A study of the official flavor criticisms of butter from 1927 to 1938, inclusive, shows that an average of 1.87 criticisms were made per sample criticized. Sixty-seven, or 79.76 per cent, of the 84 tubs of butter scored were criticized on flavor.

The distribution of the off flavors in butter are presented graphically in

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figure 1. The data show that 22.4 per cent of the flavor criticisms were "old cream"; 15.2 per cent "unclean"; 13.6 per cent "coarse"; 11.2 per cent

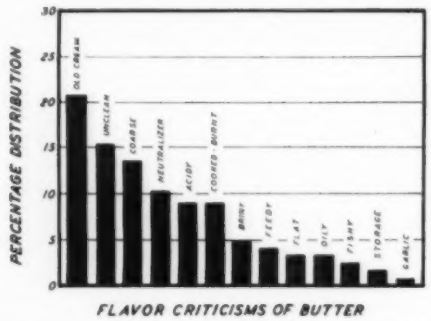

FIG. 1. Distribution of official flavor criticisms of samples of butter judged in The Students' National Contest in the Judging of Dairy Products, 1927 to 1938, inclusive.

"neutralizer"; 8.8 per cent "acidy," and 8.8 per cent "burnt," "cooked" or "heated." These six flavors accounted for 80.0 per cent of all the flavor criticisms. The remaining 20 per cent was divided among "briny," "feed," "flat," "oily," "fishy," "storage," and "garlic" criticisms, with 4.8, 4.0, 3.2, 3.2, 2.4, 1.6 and 0.8 per cent, respectively.

In the above classification some associated flavor criticisms were grouped. For example, "stale" and "cheesy" were grouped with "old cream," "bitter" with "neutralizer," "burnt" with "cooked," and so on.

Inasmuch as an average of 1.87 criticisms per sample criticized was noted, several combinations of flavor criticisms were made. Obviously, if but one criticism were used in the better grade of butter, then at least three criticisms must have been used in the poorer grades in order to maintain such an average. Combinations frequently noted were "coarse," "acidy"; "old cream," "unclean"; "neutralizer," "old cream"; and "neutralizer," "old cream," and "unclean."

The butter was scored by L. S. Edwards and G. A. Gilbert in 1927 and 1928; by L. S. Edwards and H. D. Reynolds in 1929; by H. D. Reynolds and O. A. Storvick in 1930; by H. D. Reynolds and L. D. Reekie in 1931; by C. E. Eckles assisted by coaches E. S. Guthrie and R. E. Roberts in 1932; by C. L. Pier assisted by coaches L. C. Thomsen and F. H. Herzer in 1933; and the remaining five years 1934 to 1938 inclusive by L. S. Edwards assisted each year by two of the following coaches: M. Mortensen, R. E. Roberts, S. L. Tuckey, E. S. Guthrie, L. C. Thomsen, C. M. Mecham, N. E. Fabricius, E. O. Herreid and S. T. Coulter. Thus the official judging was done during the period of this study by six official judges and 11 coach judges (1).

CHEESE

During the twelve-year period from 1927 to 1938, inclusive, 59.5 per cent of the samples of cheese used in the scoring contests were criticized for flavor by the official judges. A study of the data shows that an average of 1.38 flavor criticisms were made per cheese criticized. The distribution of those flavor criticisms is presented in figure 2. Of the many possible flavor

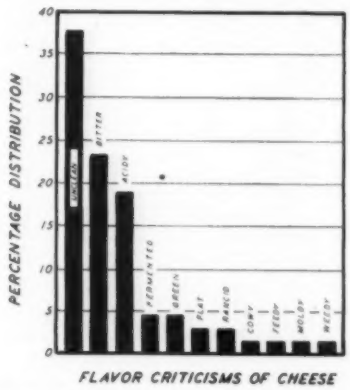

FIG. 2. Distribution of official flavor criticisms of samples of cheese judged in The Students' National Contest in the Judging of Dairy Products, 1927 to 1938, inclusive.

criticisms of cheese three seemed to command the major attention, namely, "unclean," "bitter" and "acidy," which were present 37.7, 23.2 and 18.8 per cent respectively, a total of 79.7 per cent. The remaining 20.3 per cent of the flavors was divided among eight other flavors, of which "fermented," "green," "rancid," and "flat" predominated. Such flavors as "moldy," "cowy," "feedy" and "weedy" were encountered but little in the official samples. When only one flavor criticism was made on the sample, the criticism was generally "acidy," "bitter," or "unclean." If two criticisms were made then "unclean" with either "acidy" or "bitter" were generally used.

The cheese was judged in 1927 by G. N. Tobey and G. A. Gilbert; in 1928 by G. N. Tobey, G. A. Gilbert, and L. H. Marlatt; in 1929 by H. L. Wilson and William White; in 1930 by H. L. Wilson and J. W. Moore; and in 1931 by H. L. Wilson and W. E. Ayres. Since 1931 the cheese has been judged by H. L. Wilson assisted each year by two of the following coaches: W. H. Martin, E. F. Goss, P. A. Downs, G. M. Trout, F. W. Bennett, C. A. Jacobson, R. E. Roberts, S. T. Coulter, H. G. Lindquist, W. H. Sprole, S. L. Tuckey, L. C. Thomsen, and K. R. Renner. A total of seven official judges and thirteen coach judges have placed judgments on the cheese scored from 1927 to 1938, inclusive (1).

MILK

During the twelve-year period from 1927 to 1938, inclusive, 88.1 per cent of the milk samples used in the national contests were criticized on flavor by the official judges. For each sample criticized on flavor an average of 1.27 criticisms was given. The percentage distribution of the off flavors noted in the samples is shown in figure 3. "Feed" and "cooked" flavors

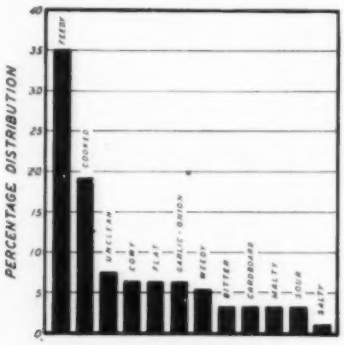

FIG. 3. Distribution of official flavor criticisms of samples of milk judged in The Students' National Contest in the Judging of Dairy Products, 1927 to 1938, inclusive.

dominated the criticisms with 35.11 and 19.14 per cent respectively, a total of 54.25 per cent. Following this group came the "unclean," "flat," "cowy," "onion" or "garlic" and "weedy" flavors, with 7.44, 6.38, 6.38, 6.38 and 5.32 per cent, respectively, a total of 31.90 per cent. The remaining 13.85 per cent was divided among "malty," "cardboard," "bitter," "sour" and "salty" flavors.

Combinations of flavors were not so readily noted in milk as in butter and cheese. When used, however, "feed" was usually one of the flavors given, the combinations being "feed," "salty"; "feed," "unclean"; "feed," "cowy"; and so on.

The milk was officially judged in 1927 by R. J. Posson; in 1928 by C. J. Babcock and R. W. Bell; in 1929 by C. J. Babcock and C. S. Leete; in 1930 by C. J. Babcock and Ernest Kelly; and in 1931 by C. J. Babcock and F. M. Grant. Since 1931 C. J. Babcock has been the official milk judge assisted each year by two of the following coaches: G. M. Trout, L. M. Thurston, L. H. Burgwald, H. G. Lindquist, E. O. Anderson, S. T. Coulter, W. H. Martin, E. L. Fouts, P. H. Tracy, F. J. Doan, T. B. Harrison and I. A. Gould. A total of 18 different judges have placed official judgments on the milk from 1927 to 1938, inclusive (1).

ICE CREAM

During the twelve-year period from 1927 to 1938, inclusive, 80.95 per

cent of the official ice cream samples in the contests were criticized on flavor. For each sample criticized on flavor an average of 2.04 criticisms were given. The percentage distribution of the flavor criticisms of ice cream during this period are shown in figure 4.

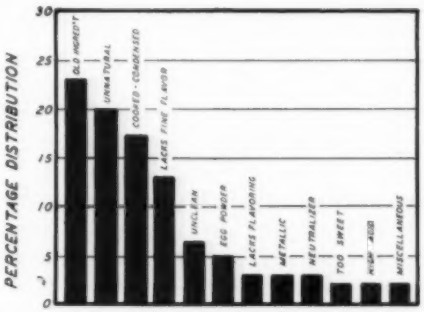

FIG. 4. Distribution of official flavor criticisms of samples of ice cream judged in The Students' National Contest in the Judging of Dairy Products, 1927 to 1938, inclusive.

Four flavors, "old ingredient," "unnatural," "condensed or cooked," and "lacks fine flavor" were noted in 23.02, 20.14, 17.26, and 12.95 per cent of the samples, respectively, a total of 73.37 per cent. "Unclean" and "egg powder" ranked next in percentage incidence with 6.47 and 5.03 per cent, respectively. The remaining 15.13 per cent was about equally divided among "lacks flavoring," "metallic," "neutralizer," "too sweet," "high acid" and "miscellaneous," being 2.87, 2.87, 2.87, 2.15, 2.15 and 2.15 per cent, respectively.

Combinations of flavor criticisms were very frequent, as shown by the fact that when a criticizable flavor was noted an average of two criticisms was given to describe it. Combinations which seemed to recur most frequently were "old ingredient" and "unnatural" or "lacks fine flavor" and "unnatural." Occasionally, another flavor criticism, "unclean," or "cooked," "condensed" or "dry milk," was used also with the combinations of the two previously mentioned.

During the period of the study the ice cream samples were officially judged one year by H. F. Judkins; two years by W. H. E. Reid; one year by P. H. Tracy; two years by A. C. Dahlberg and A. D. Burke; and the remaining seven years by A. C. Dahlberg assisted by two of the following coaches: P. H. Tracy, R. W. Smith, C. A. Iverson, E. L. Fouts, W. H. Martin, J. H. Erb, F. H. Herzer, P. S. Lucas, L. R. Dowd, P. A. Downs, G. M. Trout, and N. E. Fabricius, a total of 17 different judges (1).

SUMMARY

A study of the official flavor criticisms of butter, cheese, milk, and ice

cream samples used in the Students' National Contest in the Judging of Dairy Products during the period 1927 to 1938, inclusive, shows that relatively few flavor criticisms are used by the official judges, despite the relatively high percentage of samples criticized in that respect.

Predominating flavor criticisms of *butter* were "old cream," "neutralizer," "unclean," "coarse," "burnt" and "acidy"; of *cheese*, "unclean," "bitter" and "acidy"; of *milk*, "feed," "cooked" and "unclean"; and of *ice cream*, "old ingredient," "unnatural" and "lacks fine flavor." With the possible exception of milk, two or more criticisms were used to describe the flavor of the lower scoring samples.

The result of this study and analysis of trends in official flavor judgments is in no way intended as a guide to future scoring, but merely to classify and make available the flavor criticisms for those who may not have access to the official scoring records. Inasmuch as the samples used were selected in many cases for a specific flavor, the percentages distribution reported herein may not necessarily apply to commercial products as a whole. However, the flavor criticisms encountered in these studies appear to be representative of those encountered in the commercial products throughout the country.

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THE USE OF ANNATTO AS A TRACER IN CREAM FOR MANUFACTURING PURPOSES AND ITS DETECTION*

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Many municipalities permit the importation and sale of two grades of cream, namely, cream for fluid consumption which has been separated from milk produced under the requirements for the production of fluid milk, and cream for manufacturing purposes, produced under less stringent requirements. Cream for manufacturing purposes usually contains 40 per cent butterfat and is often shipped from distant points. On most markets cream for manufacturing purposes can be purchased cheaper than cream produced for fluid sales. For economic reasons, some milk dealers may be tempted to obtain and use cream intended for manufacturing purposes for a part or all of their fluid sales. The use of manufacturing cream for fluid purposes not only constitutes a violation of a particular ordinance, but results in unfair competition between dealers and increases the surplus over fluid sales, thus reducing prices paid producers.

The legal addition at the point of shipment of a denaturing agent or tracer to cream for manufacturing purposes would discourage the use of such cream for fluid purposes. Doan (3) reports that the State of Pennsylvania requires the addition of sugar or salt to cream and milk from uninspected sources going into the manufacture of ice cream and butter, respectively.

Alkaline annatto (vegetable cheese color)[1] is suggested as a universal tracer for addition to milk or cream intended for manufacturing purposes. The addition of 2 ml. of annatto to 10 gallons of cream is recommended. Two ml. in 10 gallons is equivalent to 1 part in 18,927. This concentration of annatto does not cause any perceptible change in color nor any change in flavor of the cream. A slight difference in color can be noted only if exceedingly careful comparisons are made. Annatto is easy to add and its presence in cream can be readily detected.

It is recommended that the annatto be added to the cream while in the pasteurizer. This method of addition permits thorough mixing and avoids the possibility of spilling annatto on the cans. Annatto may be added directly to the cream in cans. When added in this manner, considerable care

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[1] Hereinafter referred to as annatto.

must be exercised to get exactly 2 ml. in each 10 gallons and to thoroughly agitate each can after the addition of the annatto, to insure proper mixing. Care should also be exercised to prevent spilling annatto on the cans, thus causing an untidy appearance.

DETECTION OF ANNATTO

A method for the detection of annatto in milk and cream is given in the A. O. A. C., 4th Edition (4). This method has proven satisfactory on milk and on low testing cream. However, experimental work done at this station has shown this method is not satisfactory when applied to 40 per cent cream because of the high ratio of fat to casein. It was therefore necessary to devise a method for the detection of annatto in 40 per cent cream.

METHOD

Transfer 10 ml. of ethyl alcohol to a glass-stoppered flask, and add 10 ml. of the prepared cream sample. Shake vigorously for 20 seconds. Add 20 ml. of aviation grade gasoline[2] (without lead). Shake vigorously for 20 seconds. Add 0.5 ml. of formaldehyde and shake a few seconds. Transfer to a centrifuge tube and centrifuge in an unheated machine for 30 minutes at the speed (or faster) recommended for the Babcock test. By means of a pipette withdraw the alcohol-serum layer and transfer it to a small beaker. A piece of rubber tubing attached to the pipette will permit the operator to hold the tube before the eyes and thus observe when all the serum has been drawn into the pipette. Fold a 4 cm. Whatman No. 3 filter paper and place in a small (1 inch) funnel. Saturate the paper with NaOH immediately before use by placing 1 ml. of 5 per cent NaOH in the filter allowing it to run through. Filter the solution through the prepared paper. Do not allow the filter to become empty until filtration is complete, otherwise filtration will be slowed up by increased viscosity and by gumming of the filter. Open the paper and wash with a gentle stream of water. Dry the filter with or without heat. Treat paper with a few drops of a 10 per cent aqueous solution of $SnCl_2 \cdot 2H_2O$ (containing sufficient concentrated HCl to make the solution acid and a small amount of metallic tin to keep the solution reduced). Dry the paper without heat and examine by transmitted light. The appearance of any pink coloration confirms the presence of annatto. The test requires two hours to complete and is sensitive to 1 part in 500,000.

EXPERIMENTAL AND DISCUSSION

The cream used in this work contained 37 to 43 per cent fat. Ten samples of annatto as supplied by 5 manufacturers were used. Since alkaline annatto dyes the casein in the cream, the essential problem was to extract the annatto and obtain it in concentrated solution. It was also necessary

[2] 73 Octane was used in this work.

simultaneously to separate the fat to aid in breaking the emulsion, and to promote alcohol-serum separation.

Extraction of fat and annatto. On standing, or after centrifuging, the mixture will consist of three separate layers. The gasoline merely extracts the fat from solution and with the fat rises to the top of the mixture. The middle layer consists mostly of casein. The lower layer contains the alcohol, serum, and annatto if present. Obviously, annatto must not be soluble in the fat solvent, and fat must not be soluble in the annatto solvent. Fat solvents experimented with included carbon disulphide, benzene, xylene, tetra chloroethane, carbon tetrachloride, chloroform, acetone and petroleum ether. Annatto solvents experimented with included ethyl, methyl, and tertiary butyl alcohols. The solubility of annatto in a number of other alcohols was nil or slight.

Formaldehyde. The use of formaldehyde is not necessary. It does, however, harden the casein layer sufficiently to prevent it from disintegrating when inserting the pipette and thus permits easier removal of the alcohol-serum layer.

Speed of centrifuging. If time is not a factor, centrifuging is not necessary. Definite separation will occur if the mixture is permitted to stand 8 hours. When separation is accomplished by gravity, the use of a separatory funnel is recommended to permit drawing off the serum-alcohol layer. When testing cream, centrifuging 30 minutes at Babcock speed is sufficient. Higher speeds can be used satisfactorily and are necessary when testing highly stabilized ice cream mixes.

Filtration. During filtration the fibers of the filter paper absorb the annatto. The absorptive powers of different papers vary. Twenty-four grades of paper were used. Of these, Whatman No. 3 gave the best results. A 4 cm. paper is used to concentrate the annatto within a relatively small area. When milk serum is made alkaline, its viscosity increases and it becomes slightly adhesive. If the filter is permitted to become empty during filtration the sodium hydroxide in the paper tends to form an adhesive substance with the serum and to clog the paper. For most rapid filtration it is recommended that the filter be not permitted to become empty. After washing any gummy material from the filter, the paper will be dyed a straw yellow, providing annatto is present in a concentration greater than 1 part in 150,000. In greater dilutions there will be no apparent change in the color of the paper.

Drying the filter. After washing, the filter paper may be permitted to dry or if desired may be dried by means of a hot plate. However, care must be taken to prevent scorching or charring. After treatment with stannous chloride a pink coloration may be observed immediately providing the concentration of annatto was greater than 1 part in 250,000. When annatto is present in lower concentrations, it is necessary to dry the paper without heat and to examine it for traces of color by means of transmitted light. The

light source consisted of an ordinary 60 watt lamp placed 4 inches from a 3 cm. hole cut in cardboard. The color, when present, becomes more apparent after 8 to 12 hours.

Standardization of annatto. Attempts to determine a minimum standard strength of annatto for use as a tracer in cream by comparisons with a standard color solution have not proven successful. Various annatto solutions were diluted in tenth normal sodium hydroxide to the same concentration, and were compared in a Bausch and Lomb colorimeter. Some of the diluted solutions appeared identical, yet when the undiluted solutions were added to cream to make a concentration of 1 part in 500,000, it was not possible to detect the presence of certain brands of annatto. Dilute annatto solutions which appeared identical on examination in the colorimeter were then examined in a photometer. When two matched dilute solutions prepared from the same annatto solution were compared, the photometer indicated they were identical. When any two matched dilute solutions prepared from different annatto solutions were compared, the photometer indicated they were nearly identical in some instances and widely different in other instances. These results indicate that the various annatto solutions used contained at least two different dyestuffs present in different proportions. These different proportions, however, resulted in the same apparent color as determined in the colorimeter. It has also been shown by Barnicoat (1) by Carrie (2) that commercial annatto is a mixture of two or more dyestuffs and that the mixtures are not always in the same relative proportions. The substance producing positive results in the test outlined is probably the potassium salt of norbixin.

It is recommended that standardization of annatto for use as a tracer in cream be based on its detection in cream by the method outlined, the annatto to be of such strength that its presence can be detected in 40 per cent cream when diluted with 40 per cent cream to 1 part in 500,000. One of the 10 samples of annatto used could not be detected in cream when diluted with cream to 1 part in 500,000.

When annatto is added to cream in the ratio of 2 ml. to each 10 gallons, then 10 gallons of such cream would have to be mixed with more than 260 gallons of cream not containing annatto in order to produce a cream in which the annatto present could not be detected. The possibility of detecting annatto in cream in this extreme dilution would deter most "would-be" unethical milk dealers from using manufacturing cream for fluid sales.

Quantitative determination of annatto. The qualitative test for annatto can be made roughly quantitative by a comparison of the color obtained on the filter paper with colors obtained from known concentrations. When annatto is present in concentrations greater than 1 part in 100,000 the product should be diluted with cream to produce a less brilliant color, and the dilution factor used in the calculation of concentration. Dilution to the limit of sensitivity can also be applied in determining concentration.

Effect of various factors on the test. The effect of length of time of storage, freezing, acid production, neutralization, heat, and homogenization on the sensitivity of the test were studied.

Samples of cream containing various concentrations of annatto were treated as follows:

(a) Stored for a period of 2 years at 0° and 29° F., respectively.

(b) Permitted to sour until the per cent acid ranged from 0.10 to 0.80 and pH ranged from 6.6 to 4.3.

(c) Samples ranging in acidity from 0.11 to 0.80 per cent were neutralized to 0.10 per cent acid, using sodium sesquicarbonate.

(d) Heated to 190° F. and held for 10 minutes.

(e) Homogenized at 145° F. up to 4,000 lbs. pressure per square inch.

The annatto was as readily detected in all samples treated as indicated as it was in freshly prepared samples containing the same concentrations of annatto.

SUMMARY

Annatto (vegetable cheese color) is recommended as a universal tracer for use in cream intended for manufacturing purposes. A test for the detection of annatto in cream has been devised. This test is based upon the simultaneous extraction of fat with gasoline and extraction of annatto with alcohol, removal of alcohol-serum by centrifuging, absorption of annatto by an alkaline filter, and confirmation with stannous chloride. The sensitivity of the test is not affected by length of time of storage, freezing, acid production, neutralization, heat, or homogenization. The test is sensitive to 1 part annatto in 500,000 parts of cream.

ACKNOWLEDGMENT

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ADDENDA

Regulations of the Health Department of the District of Columbia as approved by the Commissioners of the District of Columbia March 7, 1939, require the addition of 4 ml. of annatto to each 10 gallons of milk, cream or ice cream mix shipped into the District of Columbia to holders of Manufacturer's Permits. Proposals for changes are now before the commissioners to reduce the amount of annatto to 2 ml. for 40 per cent cream, 1 ml. for 20 per cent cream, milk and ice cream mix.

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MILK ENERGY YIELD AND THE CORRELATION BETWEEN FAT PERCENTAGE AND MILK YIELD

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There is a considerable literature on the correlation between fat percentage and milk yield and its meaning. Krizenecky (1) compiled the published results up to 1934 for various breeds of cattle in America and Europe. He gives the following summary for 58 sets of data:

	Minimum	Maximum	Average
Variability in milk yield, V_M	11.00	31.73	21.67
Variability in fat percentage, V_f	3.83	12.69	8.58
Coefficient of correlation, r_{Mf}	−.016	−.506	−.199

The values under minimum do not necessarily, or probably, represent a single set of records; likewise under maximum. Krizenecky seems to feel that the average figures may be taken as typical of the results as found in practice for the two variables, fat percentage and milk yield.

The purpose of the present paper is to develop an estimate of the expected value of r_{Mf} on the basis of the theory that milk yield is inversely proportional to milk energy per unit milk, or milk-energy yield is independent of milk composition. It is necessary to use some approximations in this development. The purpose is not to develop a means of computing r but to develop a means of testing whether or not the constant energy theory holds good in data where the available figures are limited to the above statistical constants (plus mean fat percentage, $\bar{f}$). This, again, is not to be taken as a substitute for direct determination of the relation between fat percentage and milk-energy yield where the necessary observations are available. It is a matter of connecting fat percentage with milk-energy yield where only the statistical constants, V_f, V_M, $\bar{f}$ and r_{Mf} are given. To use Krizenecky's average figures we shall have to assume a value for $\bar{f}$ but this does not detract too seriously in the outcome.

MILK-ENERGY YIELD AND r_{Mf}

Milk energy per unit of milk may be estimated[1] as directly proportional

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[1] The estimate of milk-energy yield is commonly made in terms of 4 per cent milk (FCM) by the formula $FCM = M(.4 + .15f)$ or some modification of that formula (2, page 405). Bearing on the accuracy of the estimate may be cited 107 observations (4-week periods, Holstein cows) by the Pennsylvania Institute of Animal Nutrition in which milk-energy yield was directly determined by calorimetry. The coefficient of correlation between the determined calorie yield and FCM yield computed by the above formula from the directly determined milk and fat yields works out to be .997. The range in actual

to $2\frac{8}{9}+f$. If we have a set of records of milk yield and fat percentage for a population of cows in which the trend of milk-energy yield is horizontal when plotted against fat percentage, $r_{FCMf}=0$, the regression of milk yield on fat percentage is given by the equation,

$$M=\frac{K}{2\frac{8}{9}+f} \quad \ldots\ldots (1)$$

in which K is a constant representing the average milk-energy yield of the particular population. Equation (1) when plotted gives a curved line as illustrated diagrammatically in figure 1. The slope of the line varies throughout its course according to the equation,

$$\frac{dM}{df}=-\frac{K}{(2\frac{8}{9}+f)^2} \quad \ldots\ldots (2)$$

To the same set of records let us fit a straight line by the method of correlation. This straight line will cut the curved line of equation (1) somewhat as shown in figure 1, and its slope, S, will be,

$$S=r_{Mf}\frac{\sigma_M}{\sigma_f} \quad \ldots\ldots (3)$$

The symbol, S, is used in place of $\frac{dM}{df}$ in equation (3) to avoid possible confusion with the $\frac{dM}{df}$ of equation (2).

The slope of the two lines will be equal at or near the mean fat percentage. Let us say the slopes are equal at the mean fat percentage, then, from equations (2) and (3),

$$-r_{Mf}=\frac{\sigma_f}{\sigma_M}\frac{K}{(2\frac{8}{9}+\bar{f})^2} \quad \ldots\ldots (4)$$

In place of $\frac{\sigma_f}{\sigma_M}$ we may write $\frac{V_f\bar{f}}{V_M\bar{M}}$, in which $\bar{M}$ is the mean milk yield of the particular population. As a close approximation we may say $\bar{M}=\frac{K}{2\frac{8}{9}+\bar{f}}$, neglecting the small difference that exists as shown in figure 1. Substituting in equation (4) and rearranging we have

$$-r_{Mf}=\frac{V_f\bar{f}}{V_M}\frac{2\frac{8}{9}+\bar{f}}{K}\frac{K}{(2\frac{8}{9}+\bar{f})^2}$$

Note that K cancels out. The relation is independent of the absolute value of K, or average level of milk-energy yield in the particular population. We have then the equation,

$$-r_{Mf}=\frac{V_f}{V_M}\frac{\bar{f}}{2\frac{8}{9}+\bar{f}} \quad \ldots\ldots (5)$$

milk-energy yield per cow per day is from 1700 to 19850 calories. Holstein cows may yield as high as 38000 calories per day by 4-week periods. If the range used covered 0 to 38000 calories the coefficient of correlation would undoubtedly build itself up still closer to unity.

as an approximate expression of the correlation between fat percentage and milk yield in any set of records in which milk-energy yield is independent of fat percentage, $r_{FCMf} = 0$. Conversely, if in a certain population the observed correlation between fat percentage and milk yield is substantially that given by equation (5), the constant energy theory holds good for that population.[2]

This converse phase is of special interest because the published results of investigations on the correlation between fat percentage and milk yield often do not include a direct computation of the correlation between fat percentage and milk-energy yield, or the original data in sufficient detail for a direct computation; but the statistical constants involved in equation (5) may be included. If application of equation (5) to these constants gives a larger negative value to r_{Mf} than that found directly from the observations, it indicates a positive sign for r_{FCMf}; while a smaller negative value indicates a negative sign for r_{FCMf}. If the difference between the direct r_{Mf} and the r_{Mf} by equation (5) is small it is proof that r_{FCMf} is of small magnitude.

MILK-PROTEIN YIELD AND r_{Mf}

The relation between milk-protein yield and milk-energy yield is so nearly one of direct proportionality (3) that it is probable milk-protein yield could be substituted for milk-energy yield in the above development. Perhaps milk-protein yield is representative of the mammary machinery operating in the process of lactation, while milk-energy yield is representative of the work done by the lactating cow. The estimate of milk-protein yield from known milk and milk-fat yields is subject to greater error than is the similar estimate of milk-energy yield, and this, in the absence of direct protein determinations, favors the use of milk energy rather than milk protein.

DISCUSSION

Returning to Krizenecky's summary of 58 sets of records with respect to fat percentage and milk yield and the correlation between them, we find a range of r_{Mf} from $-.016$ to $-.506$. At one extreme is a set of records in which fat percentage and milk yield are independent ($r_{Mf} = -.016$); at the other extreme a set in which (perhaps) fat percentage and milk-fat yield are independent.[3]

[2] According to equation (5) we expect to find a larger negative value for r_{Mf} in a population of high-testing cows (as Jerseys) than in a population of low-testing cows (as Holsteins). Observed facts agree with this expectation.

By a development similar to that of equation (5) we find $r_{Ff} = \frac{V_f}{V_F} \frac{2\frac{3}{4}}{2\frac{3}{4} + \bar{f}}$. The expected (on the constant energy theory) correlation between fat percentage and milk-fat yield is positive and smaller numerically than that between fat percentage and milk yield, where $V_F = V_M$ and $\bar{f} > 2\frac{3}{4}$. Observed facts agree with this expectation.

[3] If fat percentage and milk-fat yield are independent, $r_{Ff} = 0$, we find by approximations similar to those of equation (5), $-r_{Mf} = V_f/V_M$. Krizenecky does not give V_f and

The average of the correlations is $r_{Mf} = -.20$. Applying equation (5) to the average figures (assuming $\bar{f} = 4$) we find an expected correlation of $r_{Mf} = -.24$. Using the average figures the observed and expected correlations are in good enough agreement ($-.20$ vs. $-.24$) to say that, typically, the constant energy theory holds good. The range found in r_{Mf} indicates the typical relation, is subject to a good deal of disturbance in the various sets of records as observed. These disturbances are presumably of an artificial nature, operating in either direction at random.

Stated (somewhat theoretically) another way, the populations of r_{Mf}, r_{Ff}, and r_{FCMf} each fall into a normal distribution curve; r_{Mf} with its mode at $-.24$; r_{Ff}, at $+.16$; r_{FCMf}, at zero.

As to the low value of r_{Mf}, $-.2$, equation (5) suggests that this is associated with the low ratio of variability in fat percentage to variability in milk yield. If we could eliminate the variability in milk yield due to factors other than composition of the milk and deal with a population of cows, embracing a wide range of fat percentage we might find $V_f = V_M$ and by equation (5) if $\bar{f} = 4$, expect[4] to find $r_{Mf} = -.6$.

The fact that the correlation between fat percentage and milk yield is low does not detract from its biological significance or warrant its neglect in a practical breeding philosophy.[5] For biological purposes (nutrition, ge-

V_M definitely for the particular set of records in which $r_{Mf} = -.506$ and we can only say (approximately) that $r_{Ff} = 0$ in this set of records if $V_f/V_M = .506$.

[4] In an actual case (2, page 423) dealing with average records for several years (instead of annual records) of individual cows we find $V_f/V_M = .6$ (instead of .4 as usual in annual records) and $r_{Mf} = -.44$ (expected, $r_{Mf} = -.36$). The point is that, dealing with records, as between individual cows, on the basis of the average of several years has reduced variability in milk yield and has not changed variability in fat percentage appreciably, as compared with single-year records. As expected (on the constant-energy theory) we find a relatively large observed value for r_{Mf}.

There is a limit at which equation (5) breaks down. For example, if $V_f/V_M = 2$ and $\bar{f} = 4$ equation (5) gives $r_{Mf} = -1.2$. Evidently the approximations fail us too badly at some point. However, experience with numerous actual sets of records in which r_{Mf}, r_{Ff} and r_{FCMf} are directly computed shows that equation (5) gives sensible results. Indeed, it serves as a rough check on the accuracy of the arithmetic of the computations.

[5] An adequate discussion of the genetic and other biological implications of the correlations cannot be attempted here. The commonly low observed value of r_{Mf} has given rise to the breeding philosophy that fat percentage and milk yield are independent. If such a philosophy were sound it would be very essential to breed cows giving milk of high fat percentage since, other things being equal, a 6-per-cent cow would yield as much milk as a 3-per-cent cow and twice as much milk fat! Only in a strain giving milk of high fat percentage could we hope, under such a philosophy, to attain maximum yield of milk solids or milk energy. It is true, of course, that as between various cows the yields of milk and various milk constituents (milk, water, protein, fat, lactose, ash, solids, calories) are all highly correlated one with another. If we accomplish an increase in the yield of any one we almost inevitably accomplish an increase in the yield of each of the others.

A particularly elusive feature of the relation between fat percentage and milk yield appears in dealing with the regression of fat percentage on milk yield. If $r_{Mf} = -.2$,

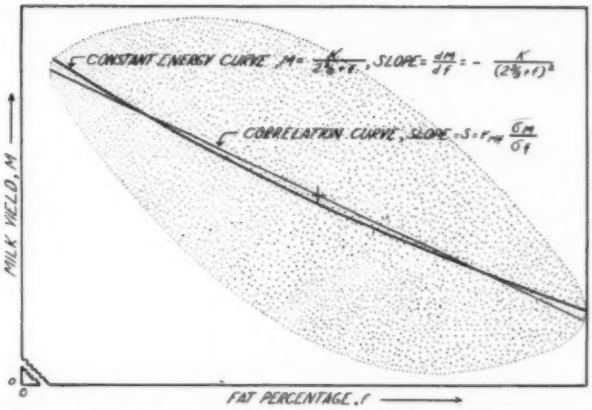

FIG. 1. Diagram in explanation of the derivation of equation (5).

The abscissas represent fat percentage; the ordinates, milk yield. The stippling represents the area of a population, but it not intended to represent density of population.

The curved regression line has the equation, $M = \frac{K}{2\frac{2}{3} + f}$. It is fitted to the individual records of the population by least squares, $K = \overline{FCM}/.15$ or $K = (20/3)\overline{FCM}$, the symbol, $\overline{FCM}$, indicating the mean of the individual FCM yields of the population. M and FCM are reckoned in the same unit of weight. All points on this curve have the same milk-energy value and hence it is referred to as a constant energy curve. The theory that this curve represents the typical regression of milk yield on fat percentage, as between comparable individual-cow records, is referred to as the constant energy theory. Milk-energy yield and FCM yield are used interchangeably, as justified in footnote 1.

The straight regression line has the equation, $M = a + \left(r_{Mf}\frac{\sigma_M}{\sigma_f}\right) f$. It is fitted to the individual fat percentage-milk yield points of the population by the method of correlation or least squares. The curve of this equation, so fitted, must go through the means of fat percentage and milk yield, marked in the diagram by a cross. It frequently happens in actual cases that the linear regression cuts $M = 0$ below $f = 10$. Obviously in such cases the linear regression will not bear extrapolation to $f = 10$. This suggests that regression within the population limits of f may be curvilinear, after the order of the constant energy curve.

The constant-energy curve, cuts the mean milk yield at the weighted mean fat percentage, $\Sigma Mf/\Sigma M$. The correlation or straight line regression cuts the mean milk yield at the unweighted fat percentage, $\Sigma f/n$ or $\overline{f}$ as symbolized. There is a small difference between the two averages and accordingly the constant energy curve does not go through the cross in the diagram.

In developing equation (5) it is assumed the two regression lines have the same slope at the unweighted mean fat percentage. It is also assumed that milk yield by the equation of the constant-energy curve at the unweighted mean fat percentage is the same as the actual mean milk yield of the particular population, neglecting the existing small difference, which amounts to less than .5 per cent of the mean milk yield. If these approximations are accepted equation (5) follows rigorously. The converse follows, if somewhat less rigorously at least with great assurance in natural data: if the correlation between fat percentage and milk yield in a particular population of records conforms to equation (5) the constant energy theory holds good for that population, that is $r_{FCMf} = 0$.

netics, etc.) we need a measure of milk yield which is independent of milk composition. Such a measure is afforded by milk-energy yield. It is not afforded by milk yield itself. It is not afforded by milk-fat yield.

SUMMARY

The theory that milk yield, so far as affected by fat percentage (or milk composition), is inversely proportional to milk energy per unit of milk (or milk-energy yield is independent of milk composition) is used to develop the expected coefficient of correlation between fat percentage and milk yield, r_{Mf}. On this theory it develops that $-r_{Mf} = \frac{V_f}{V_M} \frac{\overline{f}}{2\frac{2}{3} + \overline{f}}$, where V is variability and $\overline{f}$ is mean fat percentage. For $V_f/V_M = .4$ (the usual value as r_{Mf} has been generally derived) and $\overline{f} = 4$ the formula gives $r_{Mf} = -.24$. The low value of r_{Mf} found in practice is in keeping with the constant energy theory, and does not detract from its biological significance or warrant its neglect in a practical breeding philosophy.

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$\sigma_f = .3$ and $\sigma_M = 3000$ pounds the regression coefficient is $-.00002$, that is, an increase of 5000 pounds in milk yield is accompanied by a decrease of only .1 in fat percentage. This seems to be practically negligible.

In the biological use of the convenient term "fat percentage" it should always be held clearly in mind that fat percentage is a number expressing the average rate of milk-fat secretion, as a part, relative to the average rate of milk secretion as a whole.

BLOAT IN DAIRY CATTLE*

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The danger from bloat has been an important deterrent in pasturing legumes, especially alfalfa, red clover, and sweet clover. These legumes have proven excellent for grazing, where they can be established without too great an initial cost. The carrying capacity, palatability and effect on the flavor of milk of legumes have been favorable to their use. However, the danger of losing animals from bloat has kept many farmers from pasturing legumes more generally than is practiced at present.

Despite the fact that bloat, hoven or tympanitis has been recognized for a long time, very little experimental evidence is available as to the cause or of infallible preventive measures. A large number of preventive measures has been reported in the literature. These measures may prove successful in some communities under certain conditions, while not effective under similar conditions in other communities. Even on adjoining farms the same preventive measures may not be equally effective. Bloat may be prevalent some years and show no evidence in others, for instance for several years no bloat was experienced with cows on experimental legume pastures at South Dakota State College. The cows were fed grain, and not allowed to go on the pastures when hungry. This practice proved effective as a preventive measure against bloat for several years but in later years the cows bloated on both alfalfa and sweet clover pastures although the management of the cows had not been changed. The same pasture plots were used and the cows were fed grain before turning on the pastures as in previous years.

REVIEW OF LITERATURE

Bloat occurs more frequently on alfalfa and red clover than on sweet clover and according to some was not the same when diagnosed (1). Others have found that bloat may be caused by any kind of feed, such as spoiled silage, roots, etc. (2). When red clover is cut and fed as a soiling crop it reduces or eliminates the danger from bloat which attends its use as a pasture (3). The bloating of cattle from alfalfa in Argentina is not considered a very serious menace when rock salt is available at all times (4). Sheep are less likely to bloat on alfalfa which is mature than on young succulent growth (5). Data from 431 farms which had pastured sweet clover for an average of nine years, with 9884 cattle, 15,721 head of sheep, reported bloat

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[1] The writer is indebted to Allen Evans, and Leland Manley for conducting the gas analyses, and to Dr. G. C. Wallis for helpful suggestions in setting up the gas analyses apparatus.

occurred on 35 farms or nine per cent. The losses were no greater than might be expected from other causes. The losses were greater in irrigated and the more humid sections. Few cases of bloat were reported from pasturing the first year's growth of sweet clover (6). A survey indicated that bloat on sweet clover was worse when the young growth from the second year sweet clover is soft and succulent (7). Welch, March and Tunnicliff (8) report the results of a survey on the occurrence of bloat in sheep and cattle pastured on sweet clover. This report indicated that a thick luxuriant stand is more likely to produce cases of bloat than a scattered more stemmy growth. They found that cattlemen may expect a loss of less than 1 per cent on sweet clover pasture, and sheepmen may expect a loss of about 0.5 per cent. No particular system of managing the stock on the pasture seemed to have any influence on bloat occurrence.

Viljoens (9) defines acute tympanitis or hoven, "as an abnormal accumulation of gases in the large stomach producing great distention of the organ with subsequent paralysis of its walls." The causes are: 1, Accumulation of gases naturally formed as the result of obstruction in the gullet; 2, excessive formation and accumulation of gases in the stomach owing to causes originating in the stomach itself—(a) food stuffs easily fermented, (b) too rapid and too large consumption of green food, (c) wet, wilted, lucerne or other green food. Veech (10) states that bloat or hoven in cows is due to succulent foods eaten under certain conditions which cause the formation of large quantities of gas in the rumen or paunch, and in consequence the swelling of the left flank. It is most often seen when cattle are turned hungry on to such succulent green feed as lucerne, clover, trefoil, etc., or when cattle gorge themselves on wet grasses or herbage. He found that some cattle seem to be more subject to bloat than others. Healy and Nutter (11) attribute bloating to the sugar in the blossoms of plants. Their analysis showed that red clover blossoms contained 3.6 per cent sugar, alfalfa blossoms 2.8 per cent, and white clover blossoms 2.4 per cent, whereas the leaves of clover and alfalfa contained less than 1 per cent of sugar. When the clover blossom were ground and mixed with distilled water and held at a temperature of 37° C. for 24 hours they found an active fermentation took place. At the end of this period 45 per cent of the volume of the original clover-blossom mass of carbon dioxide, had formed. Ohman (12) found that bloat was brought about by excessive ingestion of easily fermenting foods, *e.g.*, clover, lucerne, peas, and quick growing cereal crops. Dixon (13, 14) states that, "acute bloating of ruminants, cattle particularly, may occur at any time from a variety of causes but most commonly through turning hungry cattle on to luxuriant green feed, or on to herbage country, after heavy rains and when the young herbage is making rapid growth." The editor of the "Queensland Journal" (15), in discussing bloat, attributes the cause to the filling of the paunch with gas, which causes paralyses of the muscles of the paunch, and inhibits peristalsis, which aids in belching or

expulsion of the gases from the stomach. He states, "It is possible that the cyanoglucoside content of the plant is a contributing factor to gas formation." "Hoard's Dairyman" (16), in an editorial, asserts that bloat appears more frequently in May and June and late fall, and that young animals are more subject than old animals, cattle and sheep more susceptible than horses, and hungry animals more susceptible than well-fed animals. Legumes wet with dew or rain, or frosted or wilted are likely to produce bloat. Plants grown on poor soil and low in minerals, notably lime, may be a factor in causing bloat. The editorial asserts further that, "The embryonic cells of the rapidly growing shoots of legumes contain easily fermented material, which is an important factor in bloat. Cells in the leaves are largely embryonic in nature and can be quickly broken down. If legumes are covered with hoar frost the cell walls are completely permeable. If eaten at that time, the enzyme balance is certain to be disturbed and the digestive action will start before the cell can reestablish its enzyme balance. The respiratory rate of this type of cell is very rapid and respiration is accompanied by the liberation of large quantities of carbonic acid gas." Kephart (17) after making a wide survey summed up the situation of bloat as follows:

1. Bloat is worse during May and June when the young growth from the second year sweet clover is soft and succulent.
2. Bloating is more apt to occur between 4 and 7 P.M.
3. Bloating is most apt to occur when animals are turned on pasture when hungry. Apparently they eat too greedily and the stomachs cannot take care of the large amount of material when it starts to ferment.
4. Young cattle suffer more than old ones, probably because they don't know when to stop eating.
5. Bloating seems more likely to occur when animals do not have ready access to water, or do not have an adequate quantity of minerals.
6. Cattle are principal sufferers from bloat.
7. Bloating seems to occur more frequently in some districts than others. This may be due to the lack of minerals in the soil.
8. Bloating is to some extent an individual matter with animals.
9. Bloating seems to occur more frequently in wet years than in dry years, and when plants are covered with rain or dew.

Schalk (18) used two fields of sweet clover and two fields of alfalfa. One field of each legume was equipped with a watering system with which the grass was well watered; the other was maintained under natural conditions of moisture. Comparable groups of animals were used on each type of pasture.

Before the animals were turned on the pasture, they were subjected to a number of different environmental factors such as:

1. Complete fill with dry feed in lot.
2. Complete fill with ordinary pasture (mostly bluegrass).

3. Partial fill under conditions of 1 and 2.

4. Fasted in dry lot for 12 to 18 hours and with and without water.

5. Fasted in dry lot for 12 to 18 hours and heavily salted.

6. Placed on pasture at different times of day, with heavy dew and following a rainfall.

No perceptible excessive intra-ruminal fermentation was observed in the animals placed on either pastures, following any of these preparatory environmental factors. Bloating does not appear to occur very frequently if cows are left on the pasture continually, so they never get very hungry. Other feeds in the pasture seem to reduce the danger of bloat.

Hutyra, March and Maminger (19) state, "The composition of gases in the rumen differ according to the character of the fodder and the consequent nature of the fermentations. There is always a large amount of carbonic acid gas (50 to 80 per cent of the total gas) then sulphuretted hydrogen, nitrogen and oxygen, the two last derived from the swallowed air, although oxygen is also formed during fermentation." Tappeiner (20) analyzed the gases from the rumen of an ox, two goats and a suckling lamb, and reported 45 to 67 per cent CO_2; 31–34 per cent CH_4; 0.19–7.1 per cent O_2; 0.19–4.7 per cent H_2; 1.9–15.2 per cent of N_2. Langwitz (21) analyzed ox-rumen gas and found 4 per cent O_2; 2–19 per cent N_2; 40–50 per cent CO_2 for cabbage leaf feeding, and 70–80 per cent for alfalfa, clover and grass; 16 per cent CH_4 for buckwheat and 34 per cent CH_4 after vetch feeding. Symons and Buswell (22) conducted methane fermentation on 45 substances, including carbohydrates, alcohols, acids, aldehydes, ketones, etc. It was concluded that the fermentation is an anaerobic oxidation reduction involving water and catalyzed by bacteria. The primary products are carbon dioxide and hydrogen, the two combining to form methane and water. Woodman (23), working in artificial media seeded with cultures of bacteria, has demonstrated the existence of two distinct anaerobic cellulose-fermenting organisms. The first breaks down cellulose to hydrogen and carbon dioxide, together with organic acids. The products of decomposition by the second type of organisms are methane, carbon dioxide and organic acids. Popoff (24) demonstrated in 1875 that methane formation in ruminants was attributable to micro-organisms since ruminants digest cellulose in the absence of cytase. Barker (25) suggested that methane is always formed through a reduction of carbon dioxide. Boruff and Buswell (26) studied the production of carbon dioxide and methane from anaerobic fermentation of corn stalks and suggested the equation:

$$C_6H_{10}O_5 + H_2O \rightarrow 3CO_2 + 3CH_4$$

The fermentation reaction was one of reduction and oxidation involving the addition of H_2O and forming carbon dioxide and hydrogen. Methane was formed by the combination of hydrogen with some of the carbon dioxide according to the equation:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O.$$

Fisher, Tronz, Lieske, Rudolfe (27) state that mixtures of CO and H are transformed into CH_4 in the absence of air by the action of certain bacteria (Gram positive non-spore forming bacteria).

Barker (28) in his quantitative study of methane fermentation, showed that the source of methane is always the reduction of carbon dioxide and that carbon dioxide results from the oxidation of alcohols. Christensen (29) found that alcohols, methane, hydrogen and carbon dioxide and other products are produced by the fermentation of cellulosic materials. Buswell, Boruff, Claire (30) state that methane can be produced by subjecting cellulose materials, such as corn stalks, cooked with a reagent such as lime-water to render it readily susceptible to bacterial action and then subjected to an anaerobic fermentation with bacteria such as are found in the sludge of sewage. Tappeiner (31) reported the production of 4.7 grams methane per 100 grams of cellulose digested. Kellner (32) reported 3.17 grams methane per 100 grams of starch digested, 5.45 grams CH_4 per 100 grams straw pulp, 429 grams CH_4 per 100 grams of mixed ration. Washburn and Brody (33) state, "It is generally known that considerable anaerobic carbohydrate fermentation occurs in the rumen with methane and carbon dioxide as the principal end products." The intermediary reactions whereby these end products are formed are not clearly known.

$$C_6H_{10}O_5 + H_2O \rightarrow CO_2 + CH_4$$
$$CO_2 + 4H_2 \rightarrow CH_4 + H_2O$$

Woodman and Stewart (34) assert that cellulose fermenting bacteria possess a dual mechanism whereby they first hydrolyze cellulose to glucose and then ferment the glucose to organic acids and gases. The gas-producing activities of the micro-organisms may be circumscribed by the resulting acidity of the rumen contents. Ritzman and Benedict (35) carried out experiments to determine the methane production of the horse, pig and goat. The pig produces no methane because he subsists largely on concentrates and tubers. The goat when fed on roughage produces about the same as the cow, and the horse produces about half as much.

Dougherty (36) approached the problem of bloat from the angle of type of gas produced, rather than the quantity of gas production and the subsequent intraruminal pressure. He found that 50 mm. to 90 mm. above atmospheric pressure increased blood pressure, the heart beat, and caused dyspnea, but was not the direct cause of death. Intra-ruminal pressure did, however, increase the rate of absorption of carbon monoxide and carbon dioxide from the rumen. The rumen practically ceased all movements and eructation was inhibited. These symptoms were in evidence with very low concentrations of carbon monoxide in the rumen. Hydrogen sulphide was also found in traces in the rumen gas, by Dougherty. The hydrogen sulphide appeared in greater concentrations in the liquid portion of the ingesta.

Injections of small amounts of hydrogen sulphide into the rumen of sheep proved fatal without additional pressure. Experimental evidence according to Dougherty indicates that "the concentration and the rapidity of absorption of the two toxic gases, carbon monoxide and hydrogen sulphide, from the stomach of ruminants has an important bearing on the symptoms of bloat, and in some cases may be the cause of death."

McCandlish (37), in a survey among dairy farmers in Scotland, discusses the factors which induce bloat, methods of prevention and treatment. The discussion is similar to material already reviewed. He makes the assertion that "bloat appears to be most common on the heavier and better soils of the district." He also states that "Good soils at fair elevation will generally be found to be more closely associated with bloat, than similar soils at a lower level." These conclusions are based on observations rather than experimental data.

EXPERIMENTAL

For a number of years gas from bloated cows has been collected with the thought of determining the kinds of gases and the percentage of each. It was hoped that by ascertaining the kind of gases produced, some clue to the cause of bloat might be found. Samples of gas were collected from bloated cows by means of a long rubber tube, which was inserted into the gullet and

TABLE 1

Summary of gas analyses

No. of samples	CO_2	Ill.*	O_2	H_2	CO	C_2H_4	CH_4	N_2
			A. *Gas Analysis from Cows Bloated on Sweet Clover*					
9	61.97	0.12	3.60	0.31	0.44	0.09	15.30	18.20
			B. *Generated Gas from Sweet Clover Plants*					
6	60.74	0.04	2.79	9.36	0.17	0.00	0.14	26.18
			C. *Gas Analysis from Cows Bloated on Alfalfa*					
1	59.80	0.00	3.61	0.05	0.05	0.00	18.42	18.07
			D. *Generated Gas from Alfalfa Plants*					
12	53.49	0.05	1.53	26.51	0.20	0.05	0.05	18.11
			E. *Generated Gas from Sudan Grass Plants*					
7	52.65	0.22	2.54	24.81	0.13	0.00	0.00	19.65
			F. *Generated Gas from Sorghum Plants*					
5	58.64	0.14	0.80	34.58	0.08	0.00	0.08	5.68
			G. *Generated Gas from Corn Plants*					
2	18.30	0.15	9.83	7.16	0.18	0.00	0.00	64.38
			H. *Generated Gas from Marsh or Lowland Grass*					
1	25.20	0.35	0.93	14.35	0.58	0.00	0.00	58.59
			I. *Generated Gas from Brome Grass*					
1	24.66	0.00	3.64	4.24	0.50	0.00	0.00	66.96

* Ill.—Illuminants.

thence to the rumen of the cow. Rubber gas bags and gas collecting tubes were used to collect and hold the gas until it was analyzed. The cows fought the insertion of the rubber stomach tube, and therefore some of the gas samples collected by this method were more or less contaminated with air. At other times the rumen was so filled and the pressure so great that particles of the macerated sweet clover or alfalfa were forced into the tube, necessitating withdrawing the tube to force the materials out.

Six samples of gas were secured from cows which had died from bloat. These samples were taken with a trocar and the gas collected in rubber gas bags. The gas secured in this manner was less contaminated with air than the samples of gas secured by means of the rubber tube inserted into the gullet.

A number of samples of gas were secured from legumes and non-legume plants by fermentation in the laboratory. No difficulty with bloat was experienced with non-legumes; however, it was thought advisable to ascertain the gas from these plants for comparison with the gas from plants which were known to cause bloat. The plants were collected at different stages of maturity, as well as at different times of the day, to note if that had any effect on the amount or kind of gas produced. The materials were cut, and ground with a pestle and mortar, then put in a flask and 1000 cc. of distilled water added. The generating apparatus was placed in an incubator with the temperature maintained at 37° C. When the gas container, which held a solution of 80 cc. of 3 per cent H_2SO_4 and 20 grams of Na_2SO_4 was filled with gas the glass stoppers were firmly closed and the gas stored until analyzed. The rate of generation of gas of the various plants was noted.

The Orsat apparatus for the gas analysis was used and the method of procedure outlined in Technical Bulletin 320, Department of Commerce, Bureau of Mines, was followed. A general view of the apparatus is shown on page 5 of the bulletin, therefore no attempt will be made to describe the apparatus used in the analyses. The method of procedure is also discussed completely in the bulletin, and therefore will not be outlined herein.

DISCUSSION

One sample of gas was collected from a cow which had been grazing on bluegrass pasture. The cow's horns became fastened in a woven wire fence while she was being watered, which held her muzzle under the water. She undoubtedly was forced to take in considerable water before being released, and died immediately. The gas was taken direct from the rumen, immediately after the cow died. The analysis indicated 68.12 per cent CO_2, 17.20 per cent CH_4, 12.73 per cent N_2, 1.80 per cent O_2, 0.05 per cent CO, 0.10 per cent illuminants. The analysis of the gas resulting from the conditions described above is similar to the gas analyzed from cows which bloated on sweet clover and alfalfa, yet the cow had been pasturing on a bluegrass pasture. The cause of death was not bloat, but according to the

attending veterinarian, the ingestion of large amounts of water, and finally the filling of the lungs with water.

The analysis of the gas from animals which were bloated, as well as those which had died from bloat, indicated a relatively high percentage of carbon dioxide and methane. There was no appreciable difference in the analysis of the gas samples taken with the stomach-tube and those taken by means of the trocar through the rumen wall. The difference obtained was probably due to the greater contamination from the air when the stomach-tube was used.

According to the reported analyses in tables 1A and 1C, there seems to be no significant difference in the analysis of the gas when cows bloat on sweet clover or alfalfa pastures. The number of samples of gas analyzed from alfalfa pasture is so limited that a definite conclusion on this point cannot be drawn. According to Farmers Bulletin 1653 bloat from sweet clover when diagnosed has turned out to be something entirely different than bloat from alfalfa, red clover or alsike. If this is true the difference does not appear to be in the kind of gas produced.

The analysis of gases from sweet clover and alfalfa plants, generated under laboratory conditions, shown in tables 1B and 1D, are quite similar. These plants were gathered at different times of the day as well as at different times during the pasture season. When the analysis of the gas obtained from bloated cows is compared with the analysis of gas secured from plants generated under laboratory conditions it is observed that the percentage of methane in the latter is practically negligible whereas the gas from bloated cows contains an appreciable percentage of methane. The analysis of gas from non-leguminous plants, which rarely produce bloat in cows under normal conditions, indicate no methane, except in the case of sorghum shown in table 1G.

The presence of methane in the rumen of cattle when not bloated was demonstrated as early as 1875 (24). Other workers (20, 21, 25, 31, 32, 33) have demonstrated that methane is present in the rumen of cattle and that methane results from the fermentation of cellulose materials under anaerobic conditions (22, 23, 26, 27, 28, 29, 30, 31, 33, 34).

Inasmuch as both carbon dioxide and methane are produced under normal feeding conditions in the rumen of cattle, these gases in themselves are not the direct cause of death from bloat. The carbon dioxide and methane may be responsible for the excessive pressure sustained in the rumen and as a consequence the cessation of eructation, and the normal movements of the rumen. When the gases cannot be eructed they are absorbed. The absorption of carbon dioxide and the small percentage of carbon monoxide in the rumen may prevent the hemoglobin of the blood from carrying oxygen. It is known that when carbon monoxide comes in contact with the blood by diffusion, it unites with the red pigment of the blood corpuscles, to form a relatively stable compound.

The analyses shown in tables 1B, 1D, 1E, 1F, 1G, 1H and 1I from both legumes and non-legumes under laboratory conditions show approximately the same percentage of carbon monoxide. Carbon monoxide is toxic to animals (38). It would seem, therefore, that non-legumes should produce bloat as well as legumes. The fact that cattle rarely bloat on non-legumes under normal conditions, may be due to the lower intra-ruminal pressure when non-legumes are eaten.

Dr. Dougherty in a personal letter to the writer states that in recent experimental work he has found traces of hydrogen sulphide, and this gas together with carbon monoxide in the rumen has an important influence on the symptoms of bloat, and in some cases may be the cause of death.

According to McNally (38), hydrogen sulphide acts on all animals through all tissues, especially the lungs. It produces labored breathing, pains in the stomach and death by coma. Concentrations of hydrogen sulphide above 0.1 per cent inspired air terminates in respiratory failure. In concentrations over 0.2 per cent breathing is paralyzed and asphyxia ensues.

Lange's "Handbook of Chemistry" states that "hydrogen sulphide in 0.1–0.2 per cent by volume kills most animals in a very short time."

In the gas analyses heretofore reported, no effort was made to test for hydrogen sulphide in either the gas of bloated animals or in the gas generated under specified laboratory conditions. Hydrogen sulphide results from the destruction of vegetable and animal matter; therefore, is probably present in the gases normally excreted by animals. In the case of bloat it is possible that the hydrogen sulphide is absorbed directly from the rumen similarly to the other gases, when the intra-ruminal pressure reaches a point where rumen activity is inhibited. In view of the fact (38) that low concentrations of hydrogen sulphide are fatal to animals, the absorption of it directly from the rumen may be a significant factor in the death of animals from bloat.

SUMMARY AND CONCLUSIONS

1. Analyses have been completed on gases from cows which have bloated from sweet clover and alfalfa. These gases were collected by inserting a stomach-tube into the gullet of the cows. The gases were collected in gas rubber bags, and in glass, and metal gas tubes. A number of samples of gas were collected from cows which had died from sweet clover and alfalfa bloat. These samples were collected directly from the rumen through the trocar cannula.

2. Samples of gas were collected from legume and non-legume plants generated under laboratory conditions. These samples were analyzed for the same gases as the samples from bloated animals.

3. No significant differences were indicated in the analyses of the gases from sweet clover and alfalfa plants.

4. No significant differences were discernible in the analyses of the gases

from legume and non-legume plants generated under laboratory conditions, except in the per cent of carbon dioxide in the corn plant, lowland and brome grasses. The small number of samples analyzed may account for this difference.

5. The significant difference in the analyses of the gases from bloated animals and those generated under laboratory conditions both from legume and non-legume plants, is in the methane content of the gas from bloated animals. The methane in the gas from bloated animals averaged about 17 per cent, whereas most of the samples of the gas generated in the laboratory showed no methane, and those which did indicated a negligible percentage.

6. Carbon monoxide, a toxic gas, was present in all the samples analyzed. No significant differences obtained in the per cent of carbon monoxide in the gas from different sources.

7. The percentage of gases indicated by the analyses do not in themselves point to the cause of bloat, or the cause of death from bloat. It is possible that the rapid formation and absorption of gases when legumes are eaten is due to a series of factors none of which will cause bloat or death in themselves. That is, it is known that carbon monoxide and hydrogen sulphide are highly toxic gases. These gases are produced under normal feeding conditions, in non-legumes as well as legumes yet are not fatal to the animal. The other gases present in the rumen gas are negative in their effect on the animal, yet these gases may be responsible for inducing the conditions which make the toxic gases fatal by increasing the intra-ruminal pressure or inhibiting the normal functioning of the rumen.

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ABSORPTIVE CAPACITY OF DIFFERENT MATERIALS ORDINARILY USED FOR BEDDING

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Bedding is used to keep cows clean, comfortable, and to absorb the liquid excrement. It is important, therefore, for the dairy farmer to know which materials meet these requirements at the lowest cost.

In the grain-producing sections of the United States, the cereal straws provide most of the bedding used, with shredded or cut corn stover added in the corn belt region. Shavings and sawdust are used in sections of the country where these are available at reasonable prices and where the cereal straws are high-priced and scarce. In many of the European countries such materials as peat, peat moss, and dried leaves are used. The latter products are seldom used in this country for bedding because of their scarcity and relatively higher price. The hulls of the cereals and buckwheat as well as the straws of buckwheat and flax are used as bedding in areas where these plants are grown on a commercial scale.

There is probably little difference in the "comfort effect" of the several materials used for bedding. If sufficient bedding is used any of the materials given will provide adequate comfort and protect the animals from cement surfaces or other materials which might be used for floors in stalls or stanchions.

The important consideration in beddings, therefore, is their absorptive capacity. Beddings which absorb the liquid excrement are an important factor in keeping the cows clean as well as conserving the urine, which constitutes an important part of the manurial value of the total excrement from livestock.

DISCUSSION

According to the Pennsylvania Experiment Station, the urine contains about half the nitrogen and three-fourths of the potash of the fertility elements of the manure.

Michigan circular 25 gives the following amounts of excrements voided daily by cows and the fertilizing elements contained therein:

Amount of excrement voided per day of 24 hours (1)

Solid excrements	Liquid excrements	Total excrements
lbs.	*lbs.*	*lbs.*
49	19	68

Fertilizing elements in the excrement

	Nitrogen	Phosphoric acid	Potash
	per cent	*per cent*	*per cent*
Solid excrement	0.43	0.12	0.04
Urine	1.05	trace	0.36

The daily amount and composition of solid and liquid excrement (2) voided by mature animals

	Pounds per animal		Nitrogen		Phosphorus		Potassium	
	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid
			per cent		*per cent*		*per cent*	
Cattle	52.0	19.4	3.24	0.95	0.090	0.012	0.124	0.79

Work from the South Dakota Station (3) indiacted the following amounts of solid and liquid excrements voided by lactating cows.

Average daily amount of solid and liquid excrement
(Average of 4 cows for 20 days)

Solid	Liquid
grams daily	*grams daily*
17,823.30	9072.2
(approx. 39.3 lbs.)	(approx. 20.1 lbs.)

The above cows were receiving a balanced ration of alfalfa hay and medium ground oats.

These data together with those given above, indicate the importance of conserving the liquid excrement if the farmer is to realize the manurial value from the animals maintained.

It is apparent that the manurial value of the excrement varies in amount and in its chemical constituents depending on the kinds of feed, the age and kind of animal fed. Young growing animals take more nutrients out of the feed to build bones and tissues than mature animals. A lactating cow uses considerable of the elements in the feed to produce milk. A mature animal which is not producing, or using nutrients in production, voids in the excrement a smaller percentage of the chemical elements in the feeds consumed than animals which are immature and producing.

Regardless of the varying value of the liquid excrement from cows, its value is important enough in maintaining the fertility of the farm to conserve it as well as is practically possible.

ABSORPTIVE POWER OF BEDDING MATERIALS

In five trials at the South Dakota Station (3) ten pounds whole and chopped oat, wheat and rye straws, pine shavings and sawdust were placed in a beet-pulp sack. The sacks were placed in a barrel containing sufficient

urine to completely submerge the material. Weights were placed on the sacks to assure complete covering by the urine. The sack with the material was allowed to soak for about two hours, and then suspended and allowed to drain until dripping ceased (usually from two to three hours). The materials in each sack was then emptied into a tub and weighed.

The same procedure was followed in which the bedding materials were submerged in well-water to note differences, if any, in the absorptive capacities of the two liquids. The following tables indicates the results:

*Urine absorbed by ten pounds of bedding**

Oat straw		Rye straw		Wheat straw		Shavings	Sawdust
Whole	Cut	Whole	Cut	Whole	Cut		
lbs. 17.65	*lbs.* 19.62	*lbs.* 16.43	*lbs.* 15.88	*lbs.* 17.20	*lbs.* 13.66	*lbs.* 12.10	*lbs.* 23.28

* Average of six trials.

*Water absorbed by ten pounds of bedding**

Oat straw		Rye straw		Wheat straw		Shavings	Sawdust
Whole	Cut	Whole	Cut	Whole	Cut		
lbs. 18.73	*lbs.* 19.37	*lbs.* 17.0	*lbs.* 17.23	*lbs.* 18.73	*lbs.* 18.30	*lbs.* 11.16	*lbs.* 22.40

* Average of three trials.

These data would seem to indicate there is little if any difference in the absorptive capacity of the cereal straws. The slight differences which are indicated by the data may be due to other factors, such as the moisture content of the straws used. Although the straws used appeared to be dry, it is possible that there might have been a slight difference in the moisture content. Moisture determinations were not made on the straws. The various trials were carried on over a period of several weeks. During this time the building in which the straws were soaked and allowed to drain was heated by a furnace and the humidity of the air might have varied enough to account for the differences in weight.

There seems to be no appreciable absorptive difference in whole and cut cereal straws. Inasmuch as straws are usually cut in order to make them go further, this would seem to be an erroneous idea. Inasmuch as the absorptive capacity is the important criterion on the amount of bedding to use, the cut straw would not go any further than whole straw so far as the amount of urine absorbed in concerned.

Another fallacy is that shavings will absorb more liquid than straws. These data do not indicate so. The shavings were mixed shavings and were dry, having been stored in the loft of the hay barn for several years.

The sawdust was principally from soft-wood, and drier than most sawdust used for bedding. It was secured from the local carpenter shop and had not been exposed to moisture.

The data showed no appreciable difference between the amount of urine and well-water absorbed by the cut and whole cereal straws, sawdust and shavings.

The work at Maryland (4) indicates a somewhat higher absorptive capacity than the data from the South Dakota Station excepting for sawdust which the author explained was due to the fact that the sawdust was very high in moisture. The trials were, however, conducted in a different manner, which may account for a part of the difference.

Water absorbed per pound of bedding of different materials (4)

Cut stover	Wheat straw		Shavings	Sawdust
	Whole	Cut		
lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
2.5	2.0	2.0	2.2	0.8
On basis of ten pounds, comparable to work at S.D. station				
25	20.0	20.0	22	8.0

Whisenhand (5) reports a trial in which five to seven pounds of different bedding materials were soaked in water for twelve hours, suspended in a room in the barn until dripping had ceased, and weighed after five and twenty-four hours. The following table indicates the results of the trial:

Materials	Water by 100 pounds of the material after 24 hours	Relative holding power after 24 hours
	lbs.	
Whole oat straw	240	100
Cut oat straw	244	97.6
Wheat straw	210	84.0
Mixed shavings, Chicago carload	119	47.6
Mixed shavings, from local planing mill	130	52.0
Mixed sawdust, from local planing mill	160	64.0
Fine dry, white pine shavings	185	74.0

These data indicate a somewhat higher absorptive capacity for wheat and oat straws both whole and cut. The mixed shavings data check very well with the South Dakota results. These trials were conducted similarly to the South Dakota Experiment Station trials except for length of draining and soaking time.

Herbert (6) reports the following absorptive capacities of litter:

Water retained by 100 kg. of material after 24 hours

Kinds of material	kg.
Wheat straw	220
Barley straw	285
Oat straw	228
Sawdust of poplar wood	435

These data also indicate a higher retentive capacity of the cereal straws than the data from the South Dakota Station. As previously stated, the moisture content of the straws used by the South Dakota Station was not determined. The straw was secured from stacks and was dry. The data quoted from other experimenters did not indicate whether the moisture content of the straws was known or not. It is assumed that air dried straws were used and no attempt was made to measure the moisture in the straws nor the moisture which might have been absorbed from the air.

No explanation can be offered for the lower absorptive capacities of the cereal straws used in the South Dakota trials. The time allowed (approximately two hours) for the straws to soak would seem sufficient to permit of thorough soaking, particularly when the trials were conducted in a building of approximately 60° F. temperature, and the bedding materials completely submerged. Because of the relatively lower rainfall in this territory the air-dried straw would at least be as free from moisture as straws used in the trials quoted.

The absorptive capacity of the shavings as reported in these trials checked very closely with the results from other stations.

SUMMARY

Ten pounds of oat, wheat and rye straws cut and whole were soaked from one to two hours in sufficient urine to completely submerge the sacks containing the straws. The same procedure was followed in which well-water was used instead of urine. The materials were allowed to soak for one to two hours and then suspended and allowed to drain until dripping had ceased, after which the content of the sack was emptied into a tub and weighed.

The increase in weight of the bedding materials was assumed to be due to the amounts of urine or water retained by the various materials.

No appreciable difference in absorptive capacity was noted between cut and whole straws, and straws from different cereals. Such minor differences as are indicated might be due to the completeness of soaking, or draining. The temperature and humidity of the room in which the materials were soaked and allowed to drain were not maintained at the same point, which might result in a difference in weight. That the original moisture content of the materials used for bedding affect the absorptive capacity of the materials is indicated by the sawdust used in the Maryland experiments.

The data from the South Dakota Station indicate that the whole cereal straws provide satisfactory litter. Because of the abundance and cheapness of these straws, they can be recommended in preference to any other materials for this area. The manurial value of the cereal straws is also greater than the manurial value of sawdust or shavings, materials ordinarily substituted for cereal straws.

The data did not indicate greater absorptive powers for cut straws; therefore, it would not seem advisable to cut cereal straws for bedding except where storage room for straw is at a premium.

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(5) WHISENHAND, I. W. Water holding capacities of bedding materials for live stock, amounts required to bed animals, and amount of manure saved by their use. J. Agr. Res., 14: 187–190. 1918.
(6) HERBERT, A. Farm manure. U. S. Dept. of Agr. Exp. Sta. Rec. 5: 144–158. 1893.

American Dairy Science Association Announcements

THIRTY-FIFTH ANNUAL MEETING, PURDUE UNIV., W. LAFAYETTE, IND., JUNE 24–28

HOUSING ACCOMMODATIONS

Adequate, commodious and convenient housing facilities will be available in the Cary Residence Halls and the Union Club of the Student Union Building. The Residence Halls were just completed in November, 1939, and are beautifully and pleasantly furnished with fine comfortable lounges and living quarters. The rates in the Residence Halls will be $1.25 per person per night with everything furnished. The rates for children under 12 years of age will be 75¢ per night. The rates in the Union Club which has regular hotel facilities will be from $2.50 and up per person per night. Rooms will also be available at the hotels in Lafayette.

Reservations should be made in writing to K. C. Boxell, Dairy Department, Purdue University, West Lafayette, Indiana.

REGISTRATION HEADQUARTERS

Registration Headquarters will be maintained in the lobby of the Purdue Memorial Union Building, beginning June 24, 1940.

LAST CALL FOR PAPERS

Titles and Abstracts of Papers to be presented at the June meeting must be in the hands of the Program Committee not later than April 15. Send all titles and abstracts to Dr. B. E. Horrall, Department of Dairy Husbandry, Purdue University, W. Lafayette, Indiana.

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Cornell Veterinarian

Dairy Industries
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SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BOOK REVIEWS

141. Food Control: Its Public Health Aspects: A Manual for Regulatory Officers, Food Technologists and Students of the Food Industry. JAMES HOUSTON SHRADER. Price $4.00, 513 pages. Published by J. Wiley & Sons, Inc., New York, 1939.

This book is a general text, giving a broad comprehensive view on why food control is necessary, what methods are used commercially in such control and how these control measures are applied.

Since the handling of milk is of major interest as a food control subject, almost half of the book is upon milk and milk products. The remaining parts of the book deal with meats, eggs, fish, canned foods and other products.

The author gives good evidence for the proper pasteurization of milk, ice cream mixes, cream for butter making and milk used in making the various types of cheese. The effectiveness of pasteurization was brought out in the following manner. Based on the total number of officially reported milk-borne epidemics found in the United States for the years 1923–1935 and assuming that 2 per cent of the fluid milk consumed is raw certified, 49 per cent pasteurized and 49 per cent is ordinary raw milk, it was calculated that for every outbreak due to pasteurized milk there were 2.5 due to certified and 26.3 attributable to ordinary raw milk.

This book is of value to the person interested in the general application of control measures for the various food products. The subjects covered are so broad that the author has been forced to restrict a great deal of his information to general principles and their applications. M.E.Hull

142. Standard Methods of the Division of Laboratories and Research of the New York State Dept. of Health, Second Edition. AUGUSTUS B. WADSWORTH. The Williams & Wilkins Company, Baltimore. 1939. 681 pages, illustrated, price $7.50.

This useful handbook of methods describes in detail the general and special technical procedures used in the various branches of the Division of Laboratories and Research of the New York State Department of Health. The assembly, under one cover, of the techniques and procedures employed in the various branches of the service provides a unique and servicable guide for the workers in similar fields.

This work is carefully prepared and well illustrated. The techniques and procedures are clearly described and easily followed.

Of special interest to the readers of the JOURNAL OF DAIRY SCIENCE are the sections dealing with General Laboratory Procedures; Methods Used in the Department for the Preparation of Media, Glassware, and Diagnostic

Outfits; and Methods Used in the Laboratories for Sanitary and Analytical Chemistry. T.S.S.

BACTERIOLOGY

143. Concerning Intestinal Bacteria. SIGURD FUNDER. Naturen, Nos. 7 and 8, 1939.

Mr. Funder reviews the work done in the past on intestinal microflora from the earliest days of bacteriology up to the time he conducted experiments with students at the Wisconsin University. His studies were concerned with the effect on intestinal flora in feces of adults when an exclusive diet of whole milk was used.

The experiment was carried out with three students in good health who had no previous intestinal disorders or digestive difficulties.

Contrary to previous expectations the exclusive milk diet did not change the composition of the intestinal flora. Bacterium Coli assumed the dominating position.

A complete milk diet did not create conditions that increased the lactobacilli in the intestinal canal. When large quantities of milk were taken with an ordinary mixed meal a decided increase in the lactobacilli was noted.

Added lactobacilli (*L. acidolphilus, L. bulgaricus, L. helveticus*) increased the number in feces only during the period they were added to the diet. During this period lactobacilli were not isolated from feces. This confirms the contention that an exclusive milk diet cannot give conditions for an increase of lactobacilli in the intestinal canal. Increase in lactobacilli is obtained by drinking 2 liters milk per day. An exclusive milk diet increases the pH in feces—a reduction in acidity. Joel G. Winkjer

BUTTER

144. Refrigeration in Butter and Cheese Making. L. C. THOMSEN, Univ. of Wisconsin. Refrigerating Engineering, *39:* 2, 1940.

Details of manufacture of creamery butter are outlined together with necessary refrigeration requirements in connection with pasteurization cooling loads of holding and flash pasteurization systems. Short time and long time butter storage temperatures are stated. Brief descriptions are given of the three systems of cooling applied to cream; brine, "sweet" water, and direct expansion, accompanied by tables of temperature differences to be maintained. The temperatures for storage and ripening of a number of varieties of cheese together with relative humidities are listed in tabular form. A brief discussion is devoted to freezing cheese (Cheddar) and its defrosting. The cheese should be quick frozen, maintained 10° below its freezing point temperature, and defrosted in a room maintained at a temperature of 30° F. and with low relative humidity, to prevent mold development. L.M.D.

DISEASE

145. Results from Vaccination of Heifers and Calves Against Bang's Disease. C. M. HARING, Univ. of Calif. Cert. Milk, *14:* 164, 9, 1939.

The author's experiments show that the rate of recession of agglutinins induced by vaccination is fairly rapid in calves and slower in older animals, with some animals not becoming negative again for four years or more. It is also reported that there has been no evidence of the transfer of strain 19 from vaccinated to non-vaccinated cattle which have been kept together for several years. W.S.M.

FEEDS AND FEEDING

146. The Development of Dairy Heifers. E. G. HARRISON, Cornell Univ. Cert. Milk, *14:* 162, 7, 1939.

This discussion deals chiefly with two considerations. First, what is the cost of growing a heifer from birth to the freshening age? Second, is it more economical to raise or purchase the necessary replacements? The author concludes that practically every argument favors raising the necessary herd replacements. It is pointed out that the costs of growing a replacement is so great that only heifers from profitable producing dams, and sired by good bulls should be raised. W.S.M.

147. Feeding of Grass Silage to Dairy Cows. C. B. BENDER, N. J. Agr. Exp. Sta., New Brunswick, N. J. Cert. Milk, *14:* 163, 5, 1939.

It is pointed out in this article that grass silage is a cheap priced high quality roughage, which has excellent production and growth values. Feeding trials showed that cows fed grass silage over the level of 45 pounds per day increased the color of milk because of the added carotene content. This milk was also found to be of higher quality as far as flavor is concerned. W.S.M.

FOOD VALUE OF DAIRY PRODUCTS

148. The Importance of a High Vitamin D Content in Milk. H. A. RUEHE, Univ. of Ill. Cert. Milk, *14:* 162, 5, 1939.

A review of the newer findings which have been made regarding vitamin D and their application to milk. W.S.M.

149. Measurement of the Digestibility of Milk. L. A. CHAMBERS, Univ. of Pa. Cert. Milk, *14:* 163, 2, 1939.

The various methods which have been suggested for measuring the digestibility of milk and their significance are discussed. The author briefly summarizes the present status of this subject as follows:

"The rapid development of interest in easily digested milks has created the necessity for rapid laboratory measurement of their suitability; we have certain more or less standardized tests which measure definite properties of milk products under the arbitrary test condition; but, we do not know what properties of cow's milk or its curds should be altered to produce the desired substitute for human milk, nor the conditions under which these properties should be measured." W.S.M.

ICE CREAM

150. Gone Are the Days. W. H. LIST, JR. New York, Pennsylvania and New Jersey Ice Cream Assocs. New York, N. Y. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *1:* 70, Oct., 1939.

This is an excellent historical presentation of the development of the ice cream industry. The author states that sanitation and pasteurization have played an important part in the development of the industry. The passage of state laws defining and regulating the product have been very helpful. The development of better distribution methods because of better highways and improved motor trucks has proved to be an impetus in the growth of the business. The product has been maintained in better condition by the use of dry ice and the development of dependable mechanical cabinets. Packaged ice creams, specialties and novelties have given the consumer a greater variety of good products. M.J.M.

151. The Year's Work. ROBERT C. HIBBEN, Sec., I. A. I. C. M., Washington, D. C. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *1:* 43, Oct., 1939.

The activities of the International Association of Ice Cream Manufacturers for 1939 were largely directed towards three types of considerations.

1. Federal and state regulations.
2. Inter-industry relations.
3. Association activities.

Much effort was expended in cooperation with the Federal government in an attempt to work out satisfactory standards for ice cream. Wage and hour amendments affecting the industry were made in the Fair Labor Standards Act of 1938.

Another important function of the association work is the dissemination of goodwill and correct industry information to other industries and associations in related fields of endeavor. Much of this work has been done with the cooperation of the National Dairy Council, the Dairy Industries Supply Association and the Ice Cream Merchandising Institute. M.J.M.

152. The Dairy Situation as it Affects the Ice Cream Industry. O. E. REED, Bureau of Dairy Industry, Washington, D. C. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *1:* 25, Oct., 1939.

The number of dairy cows in the United States increased from 1920 to 1934, then decreased until 1938. Since that time more than the average number of calves have been kept for replacement and because of this, the number of milch cows may increase during 1940 and 1941. Due to abundant feed supplies, milk production in 1938 reached an all-time high, with 845 pounds of milk produced for each person in the country. The total consumption of milk and milk products per person also reached a new high of 808 pounds of milk, or 37 pounds less than the per capita production.

Consumption of all dairy products is expected to increase. Ice cream consumption decreased 43 per cent from 1929 to 1933, but by 1937 had reached a level of 281 million gallons, which is considerably higher than that of 260 million gallons in 1929.

Factors which are retarding the development of the Dairy Industry are inefficient feeding and breeding methods, too low a price for milk and uneconomical disposal of by-products. At the present time much more attention is being given to the expansion of dairy markets and the utilization of skimmilk and whey by-products. These developments should affect the dairy industry favorably. M.J.M.

153. **The Ice Cream Industry in 1939.** W. J. Barritt, Poinsettia Dairy Products, Tampa, Fla. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *1:* 21, Oct., 1939.

During the past year the industry has united in working together on many pressing problems. Chief of these were in connection with the revision of the Food and Drug Act, The Motor Carrier Act and the Fair Labor Standards Act. Two tasks still ahead of the industry, according to President Barritt, are better merchandising methods and consumer education. M.J.M.

154. **Analysis of Truck Refrigerating Systems.** O'Neal M. Johnson. Int. Ass'n of Ice Cream Mfrs., Washington, D. C., Bull. 439, 1940.

In this survey, 376 ice cream plants and 48 sales branches, with a 1938 gallonage of 48,305,228 cooperated in an analysis of truck refrigeration systems. The average number of trucks per plant was $6\frac{1}{2}$; however the range in number of trucks was large because small as well as large companies were included in the survey. The results of the survey are summarized in the following table:

Ice Cream Truck Refrigerating Systems, 1939

Type of Refrigeration	Districts					
	United States	North Atlantic	Southern	Central Eastern	Mid-west	Western
	%	%	%	%	%	%
Mechanical Refrigeration:						
Continuous: Finned coil, bare tube coil, or refrigerated wall:						
Condensing Unit on truck driven only during period of delivery by:						
Mechanical power take off	0.7	0.2	0.9		4.1	0.4
Condensing Unit on truck plugged into power line at night and driven during period of delivery by:						
Generator-electric motor system	0.9	0.3	2.8		2.4	1.0
Mechanical power take off	1.9	2.1	8.3	0.8	0.7	
Holdover: Eutectic plates pulled down or frozen by:						
Central plant ammonia system	11.3	7.1	33.4	7.1	21.8	7.7
Condensing unit in garage or charging dock	13.1	17.7	4.1	15.0	9.2	8.1
Condensing unit on truck operated only at night by hook up to power line	16.2	18.4	23.4	18.3	20.8	4.2
Partial Holdover: Combining eutectic plates and expansion coils:						
Condensing Unit on truck plugged into power line at night and driven during period of delivery by:						
Separate gasoline engine	0.8	1.5	0.5		0.7	
Generator-electric motor system	1.1	0.1	0.5	0.5	7.9	
Mechanical power taken off	1.8	0.5	4.6	2.4	6.2	0.2
Non-Mechanically Refrigerated Bodies:						
Dry Ice	35.3	36.6	5.0	37.9	1.0	64.1
Cartridge, Slug, or Bullet	2.8	2.1	4.1	4.1	2.7	2.6
Cartridge combined with Dry Ice	0.2					1.0
Ice and Salt (brine)	4.4	3.6	5.5	5.7	7.2	2.8
Open or Panel Bodies:						
Used without insulation or refrigeration	5.4	1.9	6.9	7.9	14.3	5.3
With "packers"	0.5	0.3		0.3	1.0	1.0
With "packers" using frozen brine caps	0.3					1.6
Miscellaneous. Unclassified	3.3	7.6				
All trucks	100.0	100.0	100.0	100.0	100.0	100.0

M.J.M.

155. A Manufacturer Speaks of Chocolate. E. W. MEEKER, Walter Baker & Co., Inc. Ice Cream Field, *35:* 1, 21, 1940.

The author gives the results of tests carried out under the supervision of Walter Baker & Co. comparing chocolate liquor, cocoa powder and blends of these two, all of which were made from the same blend of beans. He states that the best chocolate flavor in ice cream was derived from chocolate liquor, further, that chocolate liquor was found to give the best chocolate flavor for chocolate cake coating.

It is pointed out that these results are not wholly in agreement with previously reported findings.

It is claimed in support of these results that part of the flavor is carried in the cacao fat, hence if enough chocolate liquor is used to supply the same quantity of non-fat cacao solids that would be necessary to give a desirable flavor where cocoa powder is used, that there would be more true chocolate flavor in the first than in the second case. The author states, however, that it is possible to make satisfactory chocolate flavored ice cream with either chocolate liquor, cocoa powder or a blend of the two. W.C.C.

156. Sales Advance. O'NEAL M. JOHNSON. Ice Cream Trade J., *36:* 1, 50, 1940.

The January-to-August Ice Cream Sales Index just issued by the Statistical and Accounting Bureau of the International Association of Ice Cream Manufacturers, shows for the first four months, a loss of 1.97 per cent had been sustained; in the second four, an increase of 5.08 per cent recovered earlier losses and recorded a gain for the entire eight-month period of 3.04 per cent. The Index is based on the reports of 779 plants, representing 1938 sales of 107,998,619 gallons. W.H.M.

157. Whipping Capacity of Ice Cream Mixes. B. I. MASUROVSKY. Ice Cream Trade J., *36:* 1, 52, 1940.

The author reviews some of the ice cream research work of the past year giving particular attention to the article on whipping capacity of ice cream mixes by Alan Leighton and Abraham Leviton, published in Industrial and Engineering Chemistry, Vol. 31. Reference is made to effect of aging, homogenization pressures, butterfat content, sugar, and stabilizers on the whipping properties of the mix. W.H.M.

158. Successful Retail Management. ROBERT SUTTLE. Ice Cream Trade J., *36:* 1, 36, 1940.

In addition to supervisory control and management, the retail store supervisor should inventory the store equipment at least every three months, investigate the background and education of prospective store em-

ployees, outline a training program for employees, hold weekly sales meetings, be responsible for the proper display of point-of-sale advertising and be alert for new merchandising plans and ideas. W.H.M.

159. Quality Control of Ice Cream. V. C. STEBNITZ. Ice Cream Trade J., *36:* 1, 12, 1940.

The following tests are frequently used in checking the quality of ice cream: flavor, body, texture, color, butterfat, total solids, acidity, overrun, melt-down, bacterial count, colon-aerogenes organisms, efficiency of pasteurization, and efficiency of homogenization. The author discusses each of these quality tests and how they may be used by the manufacturer in maintaining the quality of his product. The effect of ice cream ingredients on the quality of ice cream is also described. W.H.M.

160. Selective Distribution. ANTHONY MENAFRA. Ice Cream Trade J., *35:* 12, 8, 1939.

Cardani, operating in greater New York, has found that the practice of giving selected dealers exclusive agencies in a given area has resulted in higher than average prices and has increased volume consistently. This concern has specialized on the "French" type of ice cream and a "Custom-Made" pint package which is considerably richer than their bulk ice cream. They also operate a small pastry department which makes it possible to offer dealers a special ice cream and cookie combination. Spumone is also featured by this company. W.H.M.

161. The Use of Dextrose in Ice Cream. W. J. CORBETT AND P. H. TRACY. Ice Cream Trade J., *35:* 12, 11, 1939.

To test the adaptability of dextrose for use in commercial ice cream, all sucrose ice creams and part dextrose ice creams were compared. Results showed that the time required to secure 100 per cent overrun on direct expansion batch freezers was practically the same for both types of ice cream. The drawing temperature for the dextrose ice cream was about 1° F. lower than that of the sucrose ice cream. The replacement of 25 per cent of the sucrose with dextrose had little or no effect on the flavor and the body and texture scores. Dextrose ice cream melted more quickly in the mouth. The development of sandiness in high-serum-solid ice cream was delayed slightly by the dextrose. When dipped at the same temperature the loss was slightly greater in the dextrose ice cream; by lowering the dipping temperature of the dextrose ice cream the dipping losses were practically the same for both types of ice cream. At temperatures below 0° F. the penetration test was the same for both types, but above 0° F. the dextrose ice cream was less resistant. The time at which the dextrose was added made no difference in the whipping and freezing time. When frozen on a

continuous freezer the dextrose ice cream had a slightly sticky body; however, this defect could be corrected by reducing the gelatin content about one-fourth. Dextrose did not alter the color of the mix appreciably, lowered the viscosity of mix, generally lowered the pH slightly, and had no measurable effect on protein stability or curd tension of the mixes. The consumer preference study indicated that 1 pound of dextrose is equal to 0.83 pound of sucrose in sweetening ability. When one-fourth of the sucrose was replaced by dextrose at the rate of 1.43 pound to 1 pound omitted the consumers thought the dextrose ice cream tasted richer. It was also noted that the dextrose ice cream melted slightly faster on the tongue and gave a cooler, more refreshing effect. W.H.M.

MILK

162. The Route Profit and Loss Report and the Comparison Thereof. H. L. GILL, Arden Farms, Inc., Los Angeles, Calif. The Assoc. Bull., Intern. Assoc. of Milk Dealers, 32nd year, *7:* 182–184, January, 1940.

Particular attention is paid to the break-even point. It is stated that neither the total number of units nor dollars and cents sales are as good an indication of the merits of a route as the kinds of products sold. Individual route profit and loss accounting may cost no more than $1.50 per month. E.F.G.

163. How Odors and Flavors in Milk Can be Controlled by Feeding. JAMES A. EMERSON, Arden Farms, Inc., Los Angeles, Calif. The Assoc. Bull. Intern. Assoc. of Milk Dealers, 32nd year, *6:* 165–168, December, 1939.

Alfalfa hay should be fed immediately after milking and in such quantities that it is cleaned up within 3 hours. Green alfalfa should be fed twice daily in equal amounts immediately after milking is finished so that the longest time possible after feeding will elapse before the next milking. A full 5 hours should elapse between finishing roughage and milking. When silage is fed in outdoor racks with roughage, 10–12 lbs. daily right after milking seems to leave no harmful flavors or odors in the milk. Thirty or forty lbs. of silage at one feeding will not produce a fine flavored milk. Cows pastured on alfalfa, green barley, Sudan, sweet clover or rye must be put in dry corrall 5 hours before milking to produce milk free from feed odors. Mangers not cleaned frequently are responsible for off flavors in the milk. Feed flavors in milk are controlled more by the time allowance after feeding before milking is started rather than by the kind of feed used. E.F.G.

164. Rancid-Flavored Milk: Its Cause and Control. N. P. TARASSUK, Univ. of Calif., Davis, Calif. The Assoc. Bull., Intern. Assoc. of Milk Dealers, 32nd year, *6:* 153–160, December, 1939.

Practically all samples of raw milk contain a lipase or hydrolytic fat splitting enzyme. The lipase can commonly be activated by homogenization, violent shaking and by certain temperature changes. In the so-called "bitter milk of late lactation" rancidity develops naturally or without activation of any kind.

A Holstein cow that produced milk naturally lipase active gave milk of high activity on dry feed, but when changed to pasture failed to show any perceptible activity. Dry feed again produced lipase active milk, but this activity disappeared when the cow was placed on green feed again. Surface tension was decreased from 49–51 dynes in normal milk to 39–40 per cm. and occasionally lower for rancid milk. Rancidity can be detected by measuring the surface tension of the milk sooner than organoleptically. The addition of as little as 5 per cent of rancid milk to normal pasteurized whole milk produced an acid curd of weak clot and poor flavor. Heating the milk as soon as possible or not later than 4 or 5 hours after milking to a temperature of 130° F. for 30 minutes entirely prevented the development of rancid flavor within 7 days in the cold. E.F.G.

165. Electric Dairy Utensil Sterilizers. B. D. MOSES, Univ. of Calif., Davis, Calif. The Assoc. Bull., Intern. Assoc. of Milk Dealers, 32nd year, *5:* 141–150, December, 1939.

Satisfactory heat treatment of dairy utensils according to California law requires utensil exposure to at least 170° F. water or water vapor for at least 15 minutes. Two types of equipment, one a low-pressure high-wattage, sometimes called the "instantaneous type" and the other a high-pressure low-wattage one sometimes called the "steam accumulator type" are described. These are actually accessories to steam cabinets. Two drawbacks with electrically heated boilers has been hard water scale and high electric rates. The former has been remedied through water softeners and careful operation. The latter is not easily controlled but may be comparable to boilers using oil. Advantages and disadvantages of the two types of heaters are given. Descriptions include cuts of the equipment and graphs showing relationships between characteristics of equipment and current consumption. E.F.G.

166. Field Quality Control of Market Milk-Sediment Control. WALTER F. GILPIN, Golden State Co., Ltd., San Francisco, Calif. The Assoc. Bull., Intern. Assoc. of Milk Dealers, 32nd year, *5:* 135–140, December, 1939.

The author suggests doing away with a weigh can strainer—that the head on the edge of hooded pails be turned back only far enough to make a little trough which will prevent dirt from the hood entering the pail. Dust sources are drives, water, water and steam pipes and can lids (umbrella type is

recommended). The ice box is suggested as a good place to store cans. An instance is reported of an old insulated ice cream truck body being equipped with a small compressor and used as a roadside platform. In transportation a 5 ft. length of hose with a sprinkler head is suggested for washing cans. Shelter sheds are valuable for feeding hay. In one instance it was found that air currents left a dead spot at the exact location of the milk cooler allowing dust to settle. Instances of other common causes of sediment are given. E.F.G.

167. Farm Control of Milk Quality. E. H. BARGER, Borden Dairy Delivery Co., San Francisco, Calif. The Assoc. Bull., Intern. Assoc. Milk Dealers, 32nd year, *5:* 129–134, December, 1939.

A careful summary is given of the factors involved in the production of high quality milk. The producer, disease in the herd, milkers, the type and care of equipment, including milking machines and the cooling of milk are treated. The important items of laboratory reports are discussed, the author concluding with the factor of producer-distributor relations. E.F.G.

168. The American Dairy Science Association Quality Program for Dairy Products. W. H. E. REID, Univ. of Mo., Columbia, Mo. The Ass'n Bull., Intern. Assoc. of Milk Dealers, 32nd year, *5:* 121–128, December, 1939.

The author is chairman of the committee on quality program of the manufacturing section of the American Dairy Science Association. The organization of the committee and its functions during the past three years are outlined. The subcommittee on market milk has directed its attention to improvement of quality in the smaller towns and cities. Lack of uniformity of inspection and various interpretations of what constitute quality are found. A subcommittee on cream has made a study of the so-called French weed flavor. The subcommittee on butter has emphasized butter audits including analyses and keeping quality studies. The subcommittee on cheese has sponsored a program which includes control of many quality factors from the producer through to the final product. The subcommittee on ice cream has included educational ice cream scoring contests in its program. The subcommittee on condensed milk and milk powder has made a study of the quality control measures in 10 units in the industry. Many objectives and accomplishments of the quality program are pointed out and their significance suggested. E.F.G.

169. The Relation of Metals and Their Alloys to the Flavor of Milk. C. L. ROADHOUSE, Univ. of Calif., Davis, Calif. The Ass'n Bull., Intern. Assoc. of Milk Dealers, 32nd year, *6:* 161–164, December, 1939.

Attention is drawn to the fact that exposed copper and rusty equipment

are the principal causes of the objectionable flavor of pasteurized milk. The small amounts of copper sometimes required to develop oxidized flavor is shown by the fact that at the California Experiment Station when 5 gallons of hot milk was passed through a bronze sanitary pump, the tinned surface of which had been largely removed, sufficient copper was dissolved in the milk to cause a reduction in the score of two points after 4 days storage at 40° F. Rather small quantities of copper will cause oxidized flavor when the milk is especially susceptible. Tests on a number of copper-nickel alloys revealed that those containing tin and zinc lost less copper and affected the flavor of milk less than alloys not containing these metals. Some of these alloys are good for small parts of equipment which cannot be satisfactorily fabricated from non-corrodible materials. Analyses of seven alloys are given. E.F.G.

170. **The Production and Control of Good Flavor in Milk.** O. F. Garrett and C. B. Bender, New Jersey Agr. Exp. Sta. Milk Plant Monthly, *29:* 1, 23–25, 1940.

The importance of good flavor to the consumption of milk is emphasized. Placing in the hands of the consumer a fine-flavored milk is a two-fold responsibility—that of the producer and of the processor-distributor. The association between the presence of carotene and the susceptibility of the milk toward the development of the oxidized flavor suggests the necessity of feeding high carotene feeds the year round. Data show that feeds high in carotene, as grass silage, result in a milk of higher initial flavor and one more stable upon storage.

The production and feeding of grass silage appears to have several advantages: 1, the system fits into the soil erosion program through the growing of soil covering crops as legumes and grasses; 2, making of grass silage is good crop insurance as the grasses and legumes may be ensiled in wet weather; 3, ensiling grasses and legumes fit in well with the problems of producing hay; 4, the making of grass silage affords a means of making use of surplus pasture grasses; 5, ensiling grasses and legumes is a good preservation of nutrients, particularly carotene; 6, feeding grass silage results in superior flavored milk of good keeping quality; 7, grass silage of excellent quality is usually cheaper than hay of the same quality.

For winter feeding to produce milk of high color and good flavor the authors suggest the feeding of the usual grain ration, from 6–10 pounds of hay, and all the grass or legume silage the cows will eat, which ranges from 30 to 80 pounds depending on the size of the cow. They advise feeding all roughages immediately after milking, cleaning up that uneaten and ventilating the barn at least 30 minutes before each milking. G.M.T.

171. **A Comparison of the Imperviousness of Commonly Used Paper Milk Containers.** R. B. Stoltz and T. V. Armstrong, Dept. of

Dairy Techn., Ohio State University. Milk Dealer, *29:* 2, 76–82, Nov., 1939.

To determine the imperviousness of paper milk containers five different makes of quart paper milk bottles were used. Three of the makes, referred to as numbers 1, 3, and 4, are made up and paraffined at the factory. Numbers 2 and 5 are paraffined at the bottling plant just prior to filling. Numbers 1 and 5 are of square construction, the other three makes were cone shaped.

The plan of study was as follows:

One group of containers of five makes was filled with a water dye solution and held for 72 hours at 40 degrees F. and at room temperature.

A second group of containers was filled with the dye solution, held for 18 hours at storage temperatures, then hauled around the city in a delivery truck for eight hours, and returned to storage for the remainder of the 72 hours.

A third group of containers was filled with homogenized milk and held in storage for 24 hours. The dye solution was then substituted for the additional 48 hours.

A fourth group was filled with skimmilk, whole milk, 20 per cent cream, and 40 per cent cream for 72 hours.

The final part of the study was devoted to experimenting with different waxes and methods of dipping, in an effort to produce a coating that would prove entirely impervious to moisture.

From this work the authors concluded that:

"The data collected show that there is not a paper bottle on the market today that is impervious.

Our tests indicate that the two cone shaped containers, which were made up and paraffined in the factory, offered the best protection against the entrance of moisture to the fiber. Bottle No. 4 showed the least sign of absorption of the dye solution, and the least increase in weight. This was the heaviest bottle of the lot; the increased weight apparently being due to a heavier coating of paraffin. When bottle No. 3 was given an additional dipping, increasing its weight comparable with bottle No. 4, it was then as impervious to the dye as was No. 4.

While all the bottles did not show greater increases in weight when held at the higher temperatures, there is indication that increased temperature does have a tendency to cause greater absorption.

The use of 40 per cent cream seemed to render the containers less impervious than when milk or skimmilk was used.

The average weight increases of all the containers in these comparisons were, container No. 4—0.11 grams, No. 3—0.41 grams, No. 2—0.55 grams, No. 1—1.48 grams, and No. 5—1.55 grams." C.J.B.

172. Two-Quart Paper Containers on Retail Routes in New York City. ANONYMOUS. Milk Dealer, *29:* 2, 40–44, Nov., 1939.

The Sheffield Farms Company, Inc. and the Borden Farm Products Division of the Borden Company are introducing two-quart paper milk containers on retail routes in New York City at a savings to customers of three cents per bottle. The major items of saving in the new delivery system are:

Elimination of loss from lost and broken bottles—40,000 bottles per day in our operations alone (Borden).

Elimination of time and effort required to collect and handle empty bottles—approximately 25 per cent of drivers' time—and devotion of drivers' full time to selling and delivery, with less physical effort.

Elimination of expensive bottle washing machinery and its operation and maintenance.

Elimination of ice waste in refrigeration of produce on delivery vehicles. The new container can be insulated and refrigerated far more efficiently and more economically than glass bottles.

Reduction in operating cost, maintenance and depreciation of vehicles due to lighter loads. Milk in fiber containers weighs only 40 per cent as much as milk in glass. Saving in space displacement alone is more than 50 per cent.

Finally, lower prices almost automatically bring greater consumption making it possible to effect fractional savings due to increased volume.

C.J.B.

173. Observations on Cooked Flavor in Milk—Its Source and Significance. D. V. JOSEPHSON AND F. J. DOAN, Pennsylvania State College, State College, Pa. Milk Dealer, *29:* 2, 35–36, 54–62, Nov., 1939.

A detailed report of a study of the cooked flavor in milk. The authors report the following conclusions:

When milk, cream, skimmilk and some other dairy products are heated to a sufficiently high temperature or held for a sufficient period of time at lower temperatures, sulfhydryl compounds are formed from one or more of the proteins present.

These sulfhydryl compounds seem to be wholly responsible for the cooked flavor of heated milk and milk products. They are also responsible for the decrease in oxidation-reduction potential noted in heated dairy products, since they are active reducing substances.

The sulfhydryl compounds are active antioxidants and appear to be responsible for the inhibition of the development of tallowy or oxidized flavor in milk heated to temperatures over 170 degrees F.

In becoming oxidized (spontaneously or due to copper contamination) the sulfhydryls apparently lose their flavor characteristic and the milk or

milk product which previously exhibited a cooked flavor becomes indistinguishable from similar unheated milk.

Most heated milk products do not become tallowy or oxidized until the sulfhydryls are first oxidized and the cooked flavor has disappeared. Washed cream buttermilk in some cases appears to be an exception to this general rule.

The sulfhydryl substances in heated milk not only protect the milk against the development of tallowy flavor but actually act as antioxidants towards ascorbic acid (itself an antioxidant toward tallowy flavor).

Milk, cream and skimmilk heated to temperatures in excess of 170 degrees F. do not exhibit such rapid losses of ascorbic acid on storage as does raw milk or milk pasteurized in the conventional manner.

While the sources of sulfhydryl compounds have not been definitely ascertained, the lactalbumin of milk seems to be the most likely ingredient responsible, with the protein of the fat globule adsorption membrane as a possible additional source. Some constituents of the milk serum, however, seem to exercise a modifying role in the reaction brought about by the heat. C.J.B.

174. Objectives and Accomplishments of the A.D.S.A. Quality Committees of the Milk and Milk Products Industries. W. H. E. Reid, Univ. of Mo., Columbia, Mo. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *1:* 61, Oct., 1939.

The primary object of the Quality Committees of the American Dairy Science Association is to help the dairy industry to merchandise a larger volume of very high quality milk and milk products. The committee on quality are members from the faculties of the dairy departments in thirty-five states. These committees have been functioning for three years and have completed a survey of existing sanitary programs in connection with each dairy product.

The program of the sub-committee on ice cream includes the following suggestions:—formulation of standards for the ingredients used in ice cream; recommendations for sanitary control in manufacturing plants and at the point of sale; collection of statistics relative to bacteriological standards, manufacturing methods, composition, and state and municipal regulations; recommendations regarding overrun control and the preparation of a manual covering plant sanitation. Similar programs have been formulated for market milk and the principal dairy products.

The information acquired by the respective quality committees of the A.D.S.A. will be carefully studied and organized in order that it may be effectively disseminated to the industry. M.J.M.

175. The Antioxidative Action of Finely Milled Oat Flour on Milk. O. F. Garrett, New Jersey Agr. Exp. Sta. Milk Plant Monthly *29:* 2, 40, 42 and 80, 1940.

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A number of experiments were run to determine the feasibility of using oat flour, Avenex, to protect market milk against oxidative deterioration. Both glass and paper bottles were used in the study. Oat flour sprayed on the inner wall of the paper bottles as well as sprayed in the form of an oat oil, Avenol, onto the inner wall, while the paraffin was molten had a slight effect on retardation of copper induced oxidation. Likewise, when the paper was sized with oat flour before fabricating or paraffining the development of the oxidized flavor was retarded. By this method no oat flour flavor appeared in the milk and no weakening of the walls of the bottle was noted. Milk in similarly treated bottles exposed to the sun for 30 or 60 minutes did not develop the sunshine flavor despite the fact that only a small amount of oat flour was on the wall of the milk container and this under a paraffin film. G.M.T.

MISCELLANEOUS

176. **Further Development in the Modification of Cost Accounting Procedure.** E. B. McCLAIN, Assoc. Accountant, Chicago, Ill. The Assoc. Bull., Intern. Assoc. of Milk Dealers, 32nd year, *6*: 175–181. 1939.

New developments in the simplified cost accounting system particularly with reference to route operating statements and break-even points are illustrated. E.F.G.

177. **The Economic and Moral Values of Democracy.** PAUL F. CADMAN, The American Research Foundation, San Francisco, Calif. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *1:* 94, Oct., 1939.

This is an able presentation of the principles and values of Democracy as it exists in America. M.J.M.

178. **Employer-Employee Relations.** J. W. BROSTOW, Golden State Co., Ltd., San Francisco, Calif. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *1:* 84, Oct., 1939.

There are four basic factors—two or more of which are always present in any employer—employee controversy. The factors are:—

Management, which wants profit.

Labor, which wants security, high hourly wages, and recognition.

Labor unions, which want to perpetuate themselves.

Government, which would eliminate oppression.

During the past few years we have seen the results of each element struggling independently towards its objective. This situation must be replaced with techniques designed to promote cooperation between the four interested groups. Rightly or wrongly, the responsibility for engineering this cooperative approach will fall upon management. If management is

tempted to decline that responsibility, before it does so, let it pause and consider what the alternatives are apt to be. M.J.M.

179. The Bewildered American. W. C. MULLENDORE, Southern Calif. Edison Co., Los Angeles, Calif. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., *1:* 33, Oct., 1939.

This is a discussion of present economic and political questions. Suggestions are made for the solution of certain of these problems.

M.J.M.

PHYSIOLOGY

180. The Endocrinology of Milk Secretion. C. W. TURNER, Univ. of Mo. Cert. Milk, *14:* 160, 5, 1939.

Differences in productive ability of dairy cattle were found to be due to the difference in the rate of secretion of certain hormones of the pituitary and other glands of internal secretion. Production was increased by administration of dried thyroid tissue or injection of thyroxine into dairy cattle especially during the declining phase of lactation. W.S.M.

181. The Mammogenic Hormones of the Anterior Pituitary. I. The Duct Growth Factor. A. A. LEWIS AND C. W. TURNER. Mo. Agr. Exp. Sta. Res. Bull. 310, 1939.

It had been thought for a number of years that the ovarian hormones, estrogen and progestin, directly stimulated the growth of the duct and lobule-alveolar system of the mammary gland. More recently it has been shown that the action of the ovarian hormones is indirect and that the anterior pituitary secretes a mammogenic hormone which directly activates the growth of the mammary gland. There appear to be two mammogenic factors, one which stimulates the growth of the duct system and the other the lobule-alveolar system.

This paper presents the results of studies on the duct growth factor of mammogen. An assay method was developed using the male mouse. Pituitaries were collected and assayed from 545 cattle. A low level of mammogen was found in the anterior pituitaries of fetuses, growing males and steers. The mammogen content of beef and dairy anterior pituitaries was found to be low in early and late pregnancy rising to a peak at about the 150th day. Dairy cow pituitaries contained considerably more mammogen than did those of beef cows. An even higher content of hormone was found in the pituitaries of growing heifers than in pregnant females while lactatings cows had more than dry cows. During the estrous cycle the mammogen content appeared to be highest in the luteal stage.

Male rabbits given estrone responded with an increase in mammogen to double the content found in pregnant rabbits. When the estrone dosage was excessive, however, no mammogen response was secured.

Mammogen (duct growth factor) was found to be readily separated from other known anterior pituitary hormones. Any estrogen present in the anterior pituitaries was found to be far too little to have caused the mouse mammary proliferation obtained. Concentration of mammogen to 1/400 of the fresh condition was obtained. It is probable that separation of a duct-growth and a lobule-alveolar factor was secured.

Extracts of anterior pituitary were shown to develop complete mammary duct systems in male and spayed female mice, spayed female rabbits and hypophysectomized ground squirrels. Author's Abstract.